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Approche évolutive appliquée à la valorisation du potentiel en lutte biologique d'un prédateur zoophytophage, la punaise de la molène.

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Par
François Dumont

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TABLE DES MATIÈRES

LISTE DES FIGURES

LISTE DES TABLEAUX

RÉSUMÉ

Les bénéfices qu'engendre la punaise de la molène *Campylomma verbasci* (Meyer) (Hemiptera : Miridae), une espèce omnivore zoophytophage, lorsqu'elle s'alimente sur des proies (ex. acariens et pucerons) qui ravagent les cultures pomicoles peuvent être compromis par les dommages qu'elle inflige aux fruits. L'exploitation des ressources alimentaires chez les insectes zoophytophages découle d'interactions complexes entre leurs traits morphologiques, physiologiques et comportementaux, et certaines caractéristiques de l'environnement. En théorie, les individus d'une population exploitent les ressources alimentaires et investissent l'énergie et les nutriments de manière différente (ressources allouées à différents traits morphologiques et d'histoire de vie). L'exploitation des ressources alimentaires puis leur allocation à différentes fonctions biologiques par l'organisme sont déterminées, en partie, par le génotype. L'utilisation de ressources aussi différentes, que sont les proies et les plantes, peut entraîner des pressions de sélection antagonistes sur les traits impliqués dans l'omnivorie, et donc désavantager les généralistes comparativement aux spécialistes. Ainsi, selon leurs caractéristiques morphologiques, comportementales, physiologiques ou biodémographique, différents individus peuvent n'exploiter qu'une fraction des ressources totales disponibles à la population. La spécialisation de la diète serait maintenue dans la population par une hétérogénéité spatiale et temporelle de la disponibilité, de l'abondance et de la qualité des ressources. En pratique, ces différences interindividuelles au niveau de l'exploitation et de l'allocation des ressources se traduiraient par des différences au niveau de l'efficacité dans la lutte aux populations de ravageurs et également au niveau du risque que représentent les punaises de la molène pour la pomiculture. L'objectif principal de ce projet de recherche est de valoriser le potentiel en lutte biologique de la punaise de la molène en appliquant une approche évolutive qui vise l'amélioration génétique des populations. Cet objectif est divisé en sous objectifs qui amènent des connaissances nécessaires à la mise en place d'un programme d'amélioration génétique d'un agent de lutte biologique zoophytophage : 1) établir la base génétique dans la zoophagie (tétranyques et pucerons) et les traits d'histoire de vie (développement larvaire) reliés aux différences dans l'exploitation et l'allocation des ressources chez la punaise de la molène; 2) tester l'hypothèse énonçant que les différences génétiques dans la zoophagie résultent d'une spécialisation pour les ressources animales (i.e. les tétranyques) ou végétales (i.e. le pollen); 3) vérifier l'effet des différences génétiques dans la zoophagie et la spécialisation sur l'occurrence du cannibalisme; 4) finalement d'évaluer en milieu agricole les bénéfices et les risques associés à des lignées montrant une zoophagie élevée ou faible. Ultimement, ces connaissances permettront d'émettre des recommandations sur l'utilisation des punaises de la molène comme agent de lutte biologique dans les vergers de pommiers.

Douze lignées isogroupes ont été fondées à partir d'individus capturés sur le terrain à différents moments et sites au Québec. Chaque lignée était initialement composée de deux mâles et deux femelles vierges sélectionnés aléatoirement parmi les individus capturés. Ces lignées ont été maintenues pendant approximativement 15 générations (en assumant une génération tous les 40 jours). Les lignées isogroupes permettent d'augmenter le ratio entre la variabilité génétique inter-lignée (qui devrait être représentative des populations échantillonnées) et la variabilité génétique intralignée (qui devrait être réduite du fait de la dérive génique se produisant au cours du temps). Les tests de zoophagie sur les tétranyques à deux points et sur les pucerons du pêcher (deux ravageurs importants des pommiers), de développement larvaire (Chapitre II) et de choix des ressources ont été réalisés en laboratoire en utilisant les lignées isogroupes (Chapitre III). Puis deux lignées ont été sélectionnées pour leur niveau différent de voracité (un indice de zoophagie) sur les tétranyques à deux points (niveau élevé et niveau bas), puis ont été utilisées pour les tests de cannibalisme (en laboratoire) (Chapitre IV), ainsi que pour les tests en verger (Chapitre V).

En premier lieu, les bases génétiques de la zoophagie et l'effet de la disponibilité des ressources animales sur le développement larvaire ont été évaluées. Les lignées isogroupes différaient dans leur niveau de zoophagie sur les tétranyques à deux points (une proie principale) et sur les pucerons (une proie secondaire). En moyenne, le développement larvaire des punaises ne variait pas en fonction de la diète, mais les lignées avaient des temps de développement différents sans égard à la disponibilité des ressources animales. Contrairement à l'hypothèse de départ, les lignées plus zoophages n'avaient pas un développement larvaire prolongé lorsque les ressources animales n'étaient pas disponibles.

Les différences de zoophagie entre les lignées suggèrent que deux stratégies pourraient être observées chez les punaises de la molène, ce qui impliquerait un certain niveau de spécialisation soit pour les tétranyques, soit pour le pollen. Ces stratégies correspondraient aux options disponibles pour les punaises en été (sélection des hôtes herbacés avec pollen ou sélection du pommier avec tétranyques).

L'hypothèse de spécialisation alimentaire a été testée en mesurant la zoophagie des lignées en présence et en absence de pollen. Le test de choix confirme que certaines lignées de punaises favorisent les ressources animales au dépend des ressources végétales, alors que d'autres lignées réduisent considérablement leur niveau de zoophagie en présence de pollen. La forte héritabilité du choix de ressources en présence de tétranyques et de pollen appuie l'hypothèse que les prédateurs zoophytophages peuvent être plus ou moins spécialisés sur des ressources animales ou végétales. La réponse des individus aux différentes conditions écologiques pourrait varier selon leur spécialisation sur des ressources animales ou végétales.

Un lien entre le haut niveau de zoophagie et la spécialisation sur les tétranyques pourrait se refléter aussi dans le niveau de cannibalisme. Cette hypothèse a été testée sur deux lignées sélectionnées pour très forte ou très faible zoophagie. La disponibilité de tétranyques ou de pollen réduisaient le niveau de cannibalisme, mais la lignée hautement zoophage maintenait un niveau de cannibalisme moyen toujours plus élevé que la lignée peu zoophage. Cette lignée peu zoophage, spécialisée sur les ressources végétales, ne diminuait son niveau de cannibalisme (déjà faible) qu'en présence de pollen; la disponibilité des tétranyques n'avait pas d'effet sur son comportement cannibale.

Il restait à évaluer *in situ* si les lignées isogroupes sélectionnées pouvaient produire des bénéfices (contrôle des tétranyques) et/ou des désavantages différents (piqûres aux fruits). Les différences observées en laboratoire se maintenaient dans des tests en verger. Conséquemment, les lignées varient dans les bénéfices qu'elles procurent en verger; la lignée plus zoophage était plus efficace dans la lutte aux tétranyques que la lignée peu zoophage. Cependant, ces deux lignées ne variaient pas leur niveau de risque (quantité de dommages aux fruits) sur le cultivar McIntosh. Seule différence observée : les punaises hautement zoophages engendraient une chute plus importante des pommes que les punaises de la lignée peu zoophage. Ainsi, la punaise de la molène pourrait constituer un agent naturel d'éclaircissement des pommiers.

Les différences génétiques observées dans le niveau de zoophagie et dans la spécialisation pour les ressources animales ou végétales chez la punaise de la molène suggèrent que leur potentiel en lutte biologique pourrait être exploité en procédant d'abord par une amélioration génétique (par sélection artificielle des lignées très zoophages et causant peu de dommages) des populations utilisées en verger. Cette méthode *a priori* pourrait s'appliquer à toutes les espèces de prédateurs zoophytophages déjà identifiés comme agent de lutte biologique dans différents systèmes.

Mots-clés : Prédateurs zoophytophages; Lutte biologique; Génétique des populations; Miridae; Amélioration génétique; *Campylomma verbasci*.

CHAPITRE I

INTRODUCTION

1.1 La lutte biologique et les punaises zoophytophages

En milieu agricole, les ennemis naturels, tels que les prédateurs et les parasitoïdes, jouent un rôle crucial dans la réduction des dommages causés par les insectes et autres arthropodes ravageurs (Lazarovits et al. 2007; Van Driesche et Bellows 1996). La lutte biologique consiste à valoriser ces agents de lutte biologique afin de réduire les effectifs des espèces ravageuses en dessous d'un seuil de nuisibilité (Lazarovits et al. 2007; Suty 2010). Différentes approches permettent d'atteindre cet objectif à court ou à long terme; certains agents de lutte biologique s'installent dans le système et permettent un maintien durable des populations de ravageurs sous les seuils économiques, alors que d'autres agents relâchés massivement engendrent une réduction rapide des populations de ravageurs (Van Driesche et Bellows 1996). Traditionnellement, les agents de lutte plus spécifiques aux ravageurs (spécialistes de l'organisme ciblé) sont préférés aux prédateurs omnivores (Symondson et al. 2002). Cependant, les qualités des prédateurs omnivores en font des agents de luttes biologiques efficaces dans certains systèmes. Notamment, leur capacité à se nourrir de ressources alternatives (soit des proies secondaires ou de la matière végétale) leur permet de subsister dans un système en absence de leur proie principale (le ravageur à réprimer) et d'être présent dès le retour du ravageur (Coll et Guershon 2002).

L'omnivorie peut être définie de différentes façons. Pimm et Lawton (1978) définissent les omnivores en fonction de leur rôle écologique dans les interactions trophiques : est omnivore une espèce qui se nourrit de ressources à plus d'un niveau trophique. Cette définition de l'omnivorie, dite omnivorie trophique, englobe ce qu'on

pourrait qualifier d'omnivorie classique ou vraie omnivorie. Selon la définition classique d'omnivorie, une espèce se nourrit de ressources animales et végétales. Dans cette catégorie, les espèces sont classées selon un continuum dont l'extrémité dite zoophytophage regroupe les prédateurs qui consomment occasionnellement des végétaux et l'extrémité dite phytozoophage regroupe les herbivores qui consomment des proies de manière opportuniste (Coll et Guershon 2002). Cette définition de prédateurs zoophytophages est large au point où elle inclut de nombreux insectes prédateurs qui ne consomment pourtant que rarement des ressources végétales (l'inverse est aussi vrai pour les insectes phytozoophages). Pour pallier à cette lacune, Lucas et Rosenheim (2011) définissent les prédateurs zoophytophages comme des omnivores qui peuvent remplacer entièrement les ressources animales par des ressources végétales afin de compléter leur développement et leur reproduction.

Les prédateurs zoophytophages représentent un cas particulier en lutte biologique parce qu'ils peuvent s'attaquer aussi bien aux insectes ravageurs que se nourrir sur les plantes d'intérêt économique (Arnó et al. 2010; Torres et al. 2010; Castañé et al. 2011). Plusieurs punaises zoophytophages de la famille des Miridae (*Macrolophus pygmaeus* (Rambur), *Nesidiocoris tenuis* (Reuter), *Dicyphus tamaninii* (Wagner) et *Dicyphus hesperus* (Wagner) (Hemiptera : Miridae)) s'attaquent aux d'aleurodes (mouche blanche) *Bemisia tabaci* (Gennadius) et *Trialeurodes vaporariorum* (Westwood) (Hemiptera : Aleyrodidae) et aux papillons *Tuta absoluta* (Meynick) (Lepidoptera: Gelechiidae) (Albajes et Alomar 1999; McGregor et al. 1999; Lucas et Alomar 2002a; Sanchez 2008; Mollà et al. 2011; Nannini et al. 2012). Par exemple, *N. tenuis* peut réduire les populations de mouches blanches de plus de 90 % (Calvo et al. 2009). Toutefois, ces prédateurs endommagent les tomates (*Lycopersicon esculentum* Mill) en se nourrissant soit directement sur les fruits ou sur les parties végétatives du plant (Castañé et al. 2011). La quantité de dommages dépend de l'abondance relative des prédateurs et des proies (Lucas et Alomar 2002b; Calvo et

al. 2009). Les prédateurs zoophytophagous représentent donc un défi pour les entomologistes qui travaillent dans la gestion des systèmes agricoles.

La valorisation des prédateurs zoophytophages nécessite l'adoption d'approches qui permettent une augmentation des bénéfices tout en réduisant les risques de dommages à la récolte. Plusieurs aspects du comportement des punaises zoophytophages et des conséquences de leur diète sur leurs traits d'histoire de vie (ex. développement larvaire, fécondité) ont été étudiés dans le but de comprendre leur rôle en milieu agricole et leur réponse aux conditions écologiques (Gillespie et McGregor 2000; Lucas et Alomar 2002a; Sanchez et al. 2003; Sinia et al. 2004; Urbaneja et al. 2005; Hunter 2009; Moser et Obrycki 2009; Perdikis et al. 2011). Généralement, les études portant sur les punaises zoophytophages (et sur la lutte biologique) adoptent une approche populationnelle où la réponse moyenne d'une population à certains facteurs est le point clé. Cependant, il existe d'autres voies de valorisation, contrôle, utilisation des zoophytophages. La problématique des punaises zoophytophages en milieu agricole n'a jamais été abordée sous l'angle des différences génétiques, c'est l'objet de la présente thèse.

Les bénéfices et les dommages engendrés par la présence des prédateurs zoophytophages dans les zones agricoles découlent d'interactions complexes entre les traits morphologiques, physiologiques et comportementaux, et des caractéristiques de l'environnement (Albajes et al. 2006). Des différences génétiques dans ces caractères feraient varier l'efficacité des individus à consommer des ressources animales et végétales. Certains individus pourraient être plus efficaces dans la recherche, l'acquisition, la consommation et/ou la digestion des ressources animales, alors que d'autres seraient plus efficaces sur les ressources végétales. Par conséquent, certains individus spécialisés sur les ressources animales procureraient plus de bénéfices en milieu agricole, tandis que les individus spécialisés sur les ressources végétales représenteraient un risque plus élevé de dommages aux fruits ou aux plantes.

1.2 De l'approche populationnelle à l'approche individuelle

Depuis près quinze ans, beaucoup d'attention a été portée sur les différences comportementales individuelles et sur leurs conséquences dans les interactions écologiques (Dall et al. 2012). Les différences individuelles peuvent avoir des répercutions sur des processus plus larges, notamment les interactions intra- et interspécifiques, et la dynamique prédateur-proie (Dall et al. 2012; Wolf et Weissing 2012), qui sont des concepts écologiques sous-jacents à la lutte biologique (Van Driesche et Bellows 1996). Les différences individuelles peuvent particulièrement s'observer au niveau de la diète; les individus n'utilisent souvent qu'un sous-ensemble des ressources consommées par l'ensemble de la population, résultant en une spécialisation alimentaire individuelle (Figure 1.1) (Bolnick et al. 2003). Cette spécialisation survient quand les individus répondent différemment aux processus écologiques (ex. la prédation et la compétition intraspécifique) ou que leurs différences morphologiques, physiologiques et comportementales engendrent des variations dans l'efficacité à exploiter les ressources disponibles (Svanbäck et Bolnick 2005; Araújo et al. 2011).

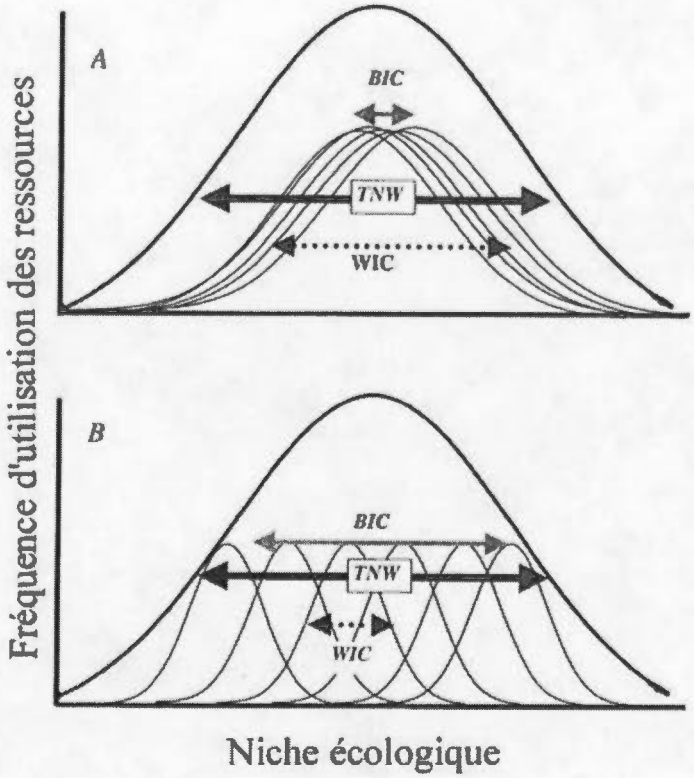

Figure 1.1. Diagramme représentant la subdivision de la niche écologique de la population entre les individus qui la composent. La taille totale de la niche (TNW de l'anglais « total niche width ») est la variance totale dans l'exploitation des ressources de tous les individus. La variation intraindividuelle de la taille des niches individuelles (WIC de l'anglais « within-individual component ») et la variance interindividuelle dans l'exploitation des ressources par les individus (BIC de l'anglais « between-individual component ») s'additionnent pour égaler TNW (TNW = WIC + BIC). Les populations A et B présentent des niches écologiques équivalentes ($TNW_A = TNW_B$). Dans la population A, les individus exploitent l'étendue des ressources disponibles ($WIC_A > BIC_A$). Dans la population B, les individus n'exploitent qu'une portion des ressources disponibles ($WIC_B < BIC_B$). (Tiré de Bolnick et al. 2003, Fig. 1).

Pour que les spécialisations alimentaires puissent évoluer en réponse aux conditions écologiques, il est essentiel qu'il y ait une base génétique à la variation phénotypique observeée pour le caractère (Roff 1992). Sokolowski (2001) soutient que le lien entre le génotype et le comportement d'approvisionnement est complexe, et dépend de l'effet de plusieurs gênes. Cette complexité peut générer des différences interindividuelles considérables dans le choix des ressources consommées. Les individus exploiteront les ressources pour lesquelles ils sont plus efficaces, reflétant leur capacité à traiter avec ces ressources (Bolnick et al. 2003). Ainsi, différents phénotypes dans la population performeraient mieux dans l'exploitation de certaines ressources et ces différences constitueraient une forme de spécialisation individuelle dans les niches écologiques (Bolnick et al. 2003).

L'exploitation de ressources aussi différentes que sont les proies animales et les plantes peut entrainer des pressions de sélection antagonistes sur les traits impliqués dans l'omnivorie. Un changement morphologique, physiologique ou comportemental qui permettrait d'améliorer les performances dans l'exploitation d'une ressource pourrait compromettre l'exploitation d'une autre (Bolnick et al. 2003; Roitberg et al. 2005). Par exemple, le stylet des hémiptères zoophages est armé de dents recurvées vers l'arrière qui permet de retenir fermement les proies (Cohen 1996). Inversement, ces dents sont recurvées vers l'avant ou absentes chez les hémiptères phytophages (Cohen 1996). Une alimentation sur des ressources végétales, qui ont la paroi cellulaire plus dure, érode les dents du stylet chez l'hémiptère zoophytophage, *D. hesperus*. Roitberg et al. (2005) observent que les individus ayant des dents érodées sont moins efficaces dans la manipulation et la consommation de proies. Ce genre de coûts associés aux changements de diète (d'une diète animale à une diète végétale par exemple) favorise l'évolution de la spécialisation alimentaire (Ma et Levin 2006).

La coexistence de différentes spécialisations alimentaires dans une même population est possible (Wilson et Yoshimura 1994). Par exemple, Jaenike et Grimaldi (1983)

ont observé que la variance génétique dans les préférences alimentaires d'une mouche polyphage, *Drosophila tripunctata* (Loew) (Diptera : Drosophilidae), n'était pas seulement significative entre des populations d'origine géographique différente, mais aussi à l'intérieur des populations. De telles différences de spécialisation seraient maintenues dans les populations par différents facteurs, notamment une hétérogénéité spatiale et/ou temporelle de l'abondance, la disponibilité et de la qualité des ressources (Wilson et Yoshimura 1994; Svanbäck et Bolnick 2005). Woo et al. (2008) observent que le comportement d'approvisionnement (c.-à.d. le temps de vol, la profondeur de la plonge et le patron de la plonge) du guillemot de Brünnich (*Uria lomvia*) (Linnaeus) varie entre les individus, et que ces différences résultent en une diète différente. Toutefois, ces spécialisations semblent équivalentes du point de vue du succès reproducteur, ce qui suggère qu'une variation dans l'abondance des proies pourrait favoriser temporairement l'une ou l'autre des stratégies (Woo et al. 2008). Robinson et Wilson (1998) proposent que des phénotypes spécialisés dans l'exploitation de ressources de faibles qualités pourraient être favorisés dans un contexte de forte compétition (ou de rareté de ressources de haute qualité). Les spécialistes des ressources de faibles qualités agiraient comme des généralistes lorsque les ressources de hautes qualités sont abondantes (Robinson et Wilson 1998). Ainsi, si les individus n'exploitent qu'une partie des niches écologiques d'une population, la compétition intraspécifique sera fréquence-dépendente et la sélection favorisera les stratégies rares si la compétition est forte (Wilson et Turelli 1986; Bolnick 2001; Araújo et al. 2009).

La spécialisation alimentaire individuelle chez les prédateurs zoophytophages pourrait théoriquement présenter un grand intérêt en lutte biologique. Des individus dans une population de prédateurs zoophytophages pourraient présenter un certain niveau de spécialisation sur les ressources animales (ex. les ravageurs à réprimer), tandis que d'autres se spécialiseraient sur les ressources végétales. Conséquemment, des individus d'une même population seraient plus bénéfiques en milieu agricole,

alors que d'autres représenteraient un risque accru de dommages aux fruits ou aux plants. Si la spécialisation alimentaire individuelle a une base génétique, il serait possible de manipuler la composition des populations de prédateurs zoophytophages afin de tirer un maximum d'avantage de ceux-ci, tout en réduisant les risques de dommages aux plantes d'intérêt économique. De plus, la variation génétique dans la zoophagie sur les proies pourraient aussi se refléter dans les interactions trophiques avec les compétiteurs soit la prédation intraguilde (quand un prédateur se nourrit d'un compétiteur) et le cannibalisme (Polis 1981; Polis et al. 1989; Rosenheim et al. 1995). Selon les conditions, la prédation intraguilde peut améliorer ou compromettre la lutte aux ravageurs (Rosenheim et al. 1995; Holt et Polis 1997; Losey et Denno 1998; Lucas et Alomar 2002a), tandis que le cannibalisme peut influencer profondément la structure et la dynamique des populations de prédateurs (Polis 1981; Claessen et al. 2004; Rudolf 2007; Torres et al. 2010). Dans le contexte des prédateurs zoophytophages, dans les périodes de faible abondance de proies, les individus pourraient se rabattre sur le cannibalisme plutôt que de consommer des ressources végétales, qui sont généralement de moins bonne qualité nutritive (Laycock et al. 2006). Ainsi, le cannibalisme chez les prédateurs zoophytophages permettrait de réduire les dommages en éliminant une partie des individus durant les périodes à haut risque (ex. quand les proies sont absentes ou rares) et en offrant une ressource alternative aux fruits et aux plants (Rudolf 2007).

1.3 La punaise de la molène

Les spécialisations alimentaires individuelles pourraient être exploitées afin de valoriser le potentiel de la punaise la molène, un prédateur zoophytophage fréquemment retrouvé dans les vergers de pommiers en Amérique du Nord (Figure 1.2) (McMullen et Jong 1970; Thistlewood et al. 1990). Cette punaise est un prédateur important d'acariens phytophages notamment des tétranyques rouges

Panonychus ulmi (Koch) et des tétranyques à deux points *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) qui causent des dommages en verger (décoloration des feuilles et défoliation des pommiers). Néanmoins, les punaises de la molène peuvent piquer dans les ovaires des fleurs de pommiers ou dans les jeunes fruits, ce qui cause des pertes économiques variables selon le cultivar (Figure 1.3) (Aubry et al. 2011). La période à risque est cependant limitée de la floraison des pommiers jusqu'au moment où les jeunes pommes atteignent un diamètre de 13 mm (soit en environ trois semaines après la floraison) (Reding et al. 2001).

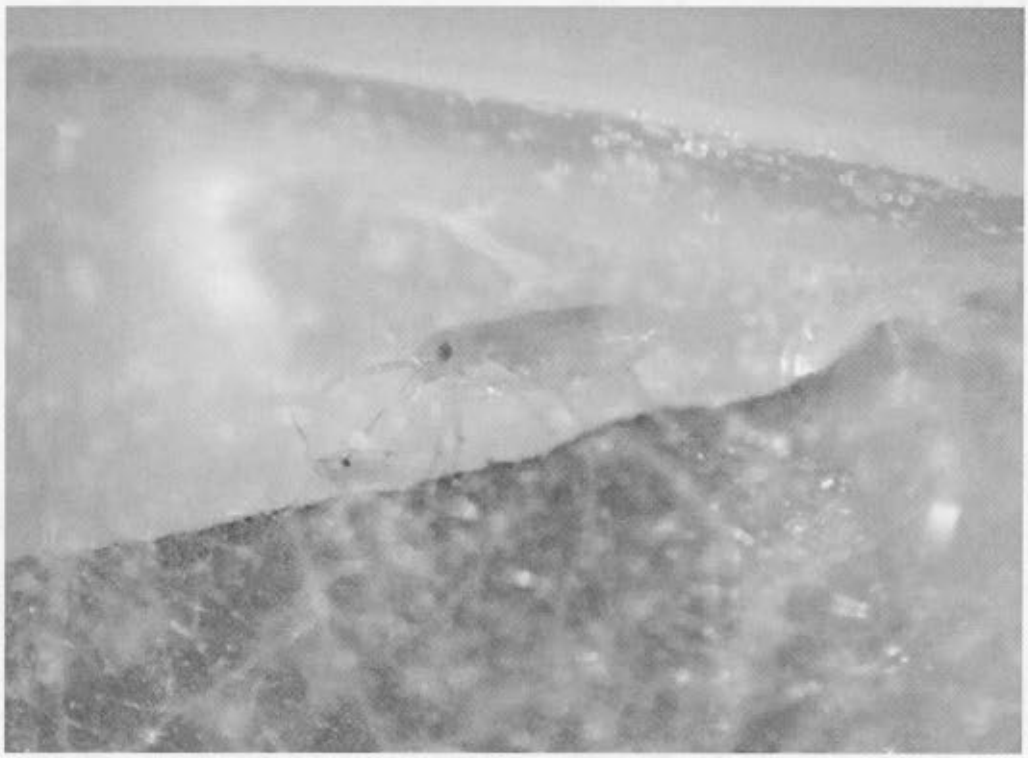

Figure 1.2. Larve de punaise de la molène (stade L4) se nourrissant d'un jeune puceron. (Photo par François Dumont).

Figure 1.3. Dommages sur une pomme causés par des piqûres de punaise de la molène alors que le fruit était en développement. (Photo par Olivier Aubry).

La punaise de la molène s'observe tôt dans la saison de production alors qu'elle émerge sur les pommiers en fleur sur lesquels elles ont passé l'hiver sous forme d'œuf (Tableau 1.1). À cette période de l'année, elle dispose de pollen qui constitue un élément important de sa diète (Bartlett 1996) et possiblement de tétranyques rouges (Parent 1973) pour compléter ces cinq stades larvaires. Les adultes de cette première génération peuvent ensuite migrer vers des hôtes herbacés alternatifs comme les plants de molènes *Verbascum thapsus* (L.) (Scrophulariaceae) ou de sumac vinaigrier *Rhus tiphina* (L.) (Anacardiacea) (Boivin et Stewart 1982; Thistlewood et al. 1990). Elles y pondront les oeufs qui engendreront la seconde génération. Néanmoins, certains adultes de première génération demeurent dans les vergers et pondent leurs oeufs dans les jeunes branches de pommiers (McMullen et Jong 1970). La proportion d'individus qui migrent ou qui restent dans le verger est inconnue, et peut certainement varier en fonction des conditions écologiques comme la disponibilité des hôtes alternatifs et des proies dans le verger. Cependant, le choix de migrer ou

non aura des conséquences importantes sur les ressources disponibles pour les larves de la seconde génération. La molène offrent une période de floraison longue et donc du pollen de manière prolongée. En revanche, ses feuilles pubescentes ne favorisent pas l'établissement d'acariens phytophages ou encore de pucerons. Ainsi, sur les plants de molène, les larves doivent essentiellement se nourrir de pollen. À l'inverse, le pollen de fleur de pommier n'est plus disponible au moment de l'émergence de la deuxième génération. Les larves sur pommier devront donc se nourrir de proies pour compléter leur développement. Ces différences écologiques importantes qui prévalent durant la deuxième génération pourraient permettre l'émergence de stratégies différentes basées sur des spécialisations pour les ressources animales et végétales.

De telles stratégies offrent une opportunité d'amélioration génétique des punaises de la molène dans le but de valoriser leur potentiel en verger de pommiers. Une utilisation estivale par le biais de lâchers inondatifs de populations sélectionnées de ce prédateur pourrait avoir un effet important sur les populations de tétranyques, tout en limitant les risques pour le développement des pommes. Avant d'effectuer de tels lâchers, des punaises très zoophages seraient sélectionnées artificiellement afin d'augmenter leur efficacité en verger et d'augmenter leur spécificité pour les proies ciblées.

Tableau 1.1. Stades de développement de la punaise de la molène et présence des proies en rapport avec le développement des pommiers. (Adaptation de Chouinard et al. (2001)).

	Hiver	Débourre-ment	Bouton rose	Floraison	Calice	Juin	Juillet	Août	Récolte
Punaise de la molène									
Oeufs	———	———	———	———			- - - - -		
Larves			———	———	———	———	- - - - -	- - - - -	
Adultes					———	———	- - - - -	———	———
Principales proies									
Tétranyque rouge			———	———	———	———	———	———	———
Puceron vert						———	———	———	
Tétranyque à deux points						———	———	———	———

Légende :

——— Activité sur pommier

- - - - - Activité sur molène ou autres herbacées

1.4 Structure de la thèse, objectifs et prédictions

Ma thèse présente l'idée que le potentiel des punaises de la molène en lutte biologique peut être valorisé en sélectionnant artificiellement les individus les plus bénéfiques pour la pomiculture, en fonction de leurs spécificités alimentaires zoophytophages. Elle s'appuie sur l'hypothèse que la variation des spécialisations alimentaires pour les ressources animales ou végétales, observée chez la punaise de la molène, a une base génétique, et peut engendrer des différences notables aux niveaux des bénéfices et des risques que procure ce prédateur en milieu agricole.

Dans un premier temps, je présenterai une étude sur les bases génétiques de la zoophagie des punaises de la molène sur les tétranyques et les pucerons, des traits d'histoire de vie (développement larvaire) en réponse à la disponibilité des proies et des corrélations génétiques entre la zoophagie et le développement larvaire chez la punaise de la molène. Cette étude constitue le chapitre II sur lequel repose tous les autres chapitres. L'utilisation de douze lignées isogroupes, chacune fondée à partir de deux couples, a permis d'estimer la variation génétique dans les caractères étudiés. Je m'attends à une association génétique entre le niveau de zoophagie et les performances en terme de traits d'histoire de vie; les lignées plus zoophages réduisent le temps de développement larvaire en présence de proie, tandis qu'elles sont moins performantes (développement prolongé) que les lignées peu zoophages lorsque les proies ne sont pas disponibles.

Au chapitre III, l'hypothèse que le niveau de zoophagie des lignées est négativement corrélé avec leur niveau de phytophagie (hypothèse de spécialisation alimentaire) a été testée. Dans cette étude, le comportement de zoophagie des punaises sur les tétranyques à deux points a été observé en présence et en absence de pollen, une ressource végétale riche en protéine. Ainsi, je prédis que si les punaises de la molène

ont un certain degré de spécialisation alimentaire pour un type de ressources, les lignées se spécialisant sur des ressources animales ne modifient pas leur comportement en fonction de la disponibilité du pollen, alors que les lignées spécialisées sur les ressources végétales changent leur diète pour inclure leurs ressources préférées.

Dans la chapitre IV, je compare l'intensité du cannibalisme de deux lignées, la plus zoophage et la moins zoophage, en réponse à la disponibilité de proies ou de pollen. Mes prédictions sont : 1) que la lignée la plus zoophage est plus cannibale que la lignée moins zoophage; et 2) qu'elle réduit son niveau de cannibalisme uniquement lorsque les proies sont disponibles. Inversement, la lignée la moins zoophage réduit son degré de cannibalisme lorsque le pollen est disponible.

Au chapitre V, des tests sont menés en verger de pommiers, sur les deux lignées la plus et la moins zoophages, afin de valider les hypothèses suivantes : 1) la lignée la plus zoophage engendre moins de dommages aux fruits qu'une lignée la moins zoophage; et 2) la lignée la plus zoophage est plus efficace dans la lutte aux tétranyques que la lignée la moins zoophage. Les dommages aux pommes ont été évalués durant la période à risque (de la floraison jusqu'à ce que les fruits atteignent 13 mm), tandis que l'efficacité des lignées dans la lutte aux tétranyques est estimée en fin d'été (avant la récolte).

CHAPITRE II

GENETIC BASIS OF ZOOPHAGY AND NYMPHAL DEVELOPMENTAL TIME IN ISOGROUP LINES OF THE ZOOPHYTOPHAGOUS MULLEIN BUG, *CAMPYLOMMA VERBASCI*.

Dumont, F., Lucas, E. & Réale, D.

2.1 Abstract

Zoophytophagous predators can substitute phytophagy for zoophagy to complete their development and reproduce. Individuals within a zoophytophagous species could differ in their efficiency to exploit either animal or plant resources. To evolve the efficiency of zoophagy must have a heritable basis. However, given the relative specializations to each type of diet, there may be a trade-off between fitness performances on a carnivorous and on a plant diet, such that the most efficient genotypes on a carnivorous diet perform poorly on a plant diet. Such a trade-off may have important consequences on the efficiency of zoophytophagous species as biocontrol agents. In this study, we estimated the genetic basis for zoophagy and nymphal development length and the genetic correlation between these two traits, in the mullein bug, *Campylomma verbasci* (Meyer) (Hemiptera: Miridae), a species commonly found in Canadian apple orchards. In the laboratory, we counted the number of two-spotted spider mites, *Tetranychus urticae* (Koch) (Acarina: Tetranychidae), and green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), killed in 24 hours by nymphs and by adults of 12 isogroup lines. We also studied nymphal development length of the isogroup lines under 1) a purely plant, and 2) a mixed diet. We showed among-isogroup-line variance in the level of

zoophagy (i.e. number of spider mites and aphids killed), and a positive among-line correlation between them. Diet had no significant effect on the mean nymphal development length, but isogroup lines differed significantly in nymphal development regardless the diet. Our results reveal genetic differences in foraging efficiency on prey, which suggest that some genotypes in population of the zoophytophagous mullein bug could provide more benefits in apple orchards. We suggest that artificial selection on the level of zoophagy could improve the efficiency of zoophytophagous predators, and discuss its consequences on the role of zoophytophagous predation in crop systems.

2.2 Introduction

In many organisms, specializing on a given resource could decrease the efficiency of foraging on alternative resources (Bolnick et al. 2003). Individuals may exploit different subsets of the population's food resources, resulting in diet variation among individuals in a population (Bolnick et al. 2003). As a consequence individuals within a population may not be considered as ecologically equivalent (Bolnick et al. 2003, 2011; Sih et al. 2012). This is the case of zoophytophagous predators that feed preferentially on prey, but can substitute or complement prey with plant resources (Coll and Guershon 2002; Arnó et al. 2010). Plant and prey resources vary in space and time for a wide range of characteristics (e.g. chemical composition, nutritional value, defence strategies, distribution, availability) (Cohen 1996; Coll and Guershon 2002). Such variation may generate a trade-off between foraging efficiency on different types of resource. Trade-offs can occur in basically all the aspects of foraging behaviour such as resource recognition, capture, handling and digestion (Coll and Guershon 2002; Cohen 1996; Boyd et al. 2002; Roitberg et al. 2005). Consequently instead of being composed of highly plastic genotypes that can easily switch from a zoophagous to a phytophagous diet, zoophytopagous populations may

be composed of a mix of specialised genotypes that rely mostly on prey ("zoophagous stragegy"), or on plant resources ("phytophagous strategy"), and of generalists that show variable plasticity levels in the resource they consume ("zoophytophagous strategy"). We should thus find genetic differences and a continuous variation in the diet preference within a population.

Among-individual variation in the diet may have noticeable consequences on life-history traits and thus on fitness. Compared to a diet exclusively composed of either plant or animal resources, a mixed-diet generally improves adult and nymph survivorship, development time, fecundity and longevity (Naranjo and Gibson 1996; Lemos et al. 2010). A poor diet deprived of one type of resource may generate a trade-off between traits (Stearns 1989; Legaspi et al. 1996). For instance, when exclusively fed on tomato fruits (*Solanum lycopersicum*), *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) nymphs decreased their mortality rate and extended their development time compared to nymphs that consumed pure animal-based diet (i.e. whiteflies, *Ephestia* eggs, aphids and *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) nymphs) (Lucas and Alomar 2001). Furthermore, nutrients can have different effects on different life-history traits (Catoni et al. 2008). An unbalanced diet caused by temporal and spatial changes in resource and nutrient availability, coupled with individual variation in food preferences, may result in life-history differences and trade-offs among individuals (Catoni et al. 2008).

The evolution of such life-history/foraging strategies requires genetically based among-individual variation in the diet (Roff 1992; 2002). Genetic differences in foraging behaviour have been shown to generate variation in diet in a wide range of arthropod species (Jaenike and Holt 1991). This variation may potentially be related to life-history variation. For instance, two strains of *Drosophila tripunctata* Loew (Diptera: Drosophilidae) consistently exhibited preferences for different types of food resources (Jaenike 1985). Similarly, a strong genetic basis for both prey finding and consumption, and a quick response of these traits to artificial selection has been found for the predatory mite *Phytoseiulus persimilis* (Evans) (Nachappa et al. 2010). In this

study selection on consumption rate induced change in development time and fecundity; low-consumption strains having a longer development time and a lower fecundity than high-consumption strains (Nachappa et al. 2010). In contrast direct evidence for genetic variation in foraging behaviour and their effects on life-history traits has yet to be found in zoophytophagous predators.

In the present study, we tested whether the zoophytophagous mullein bug *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) showed genetic variation in zoophagy and whether genotypes with different levels of zoophagy adjusted their nymphal development differently when they experience a pure plant-based or a mixed animal-plant diet. The mullein bug is largely distributed among North American apple and pear orchards (McMullen and Jong 1970; Thistlewood et al. 1990; Arnoldi et al. 1992). In apple orchards, it is one of the most important predators of the European red spider mite *Panonychus ulmi* (Koch), the two-spotted spider mite *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) and, to a lesser extend, the apple aphid *Aphis pomi* (de Geer) (Hemiptera: Aphididae) (McMullen and Jong 1970; Thistlewood et al. 1990; Arnoldi et al. 1992). Mullein bugs emerged early in the growing season, and are synchronized with both the flowering of apple trees and red spider mites emergence. Thus, spring nymphs feed mainly on pollen, red spider mites, and developing apple fruits (Thistlewood et al. 1990; Arnoldi et al. 1992).

We estimated the genetic basis of the number of spider mites or aphids killed per individual bug per day, and the genetic correlation between these traits and nymphal development length using isogroup lines. The occurrence of a trade-off between development length in mixed diet and on a purely phytophagous diet may have affect both the efficiency of mullein bugs to control for pests and their ability to damage apples.

2.3 Materials and Methods

2.3.1 Mullein bug and prey populations

Mullein bugs were captured in the field as eggs, nymphs, or adult stages in different regions of Québec (Canada). In summer 2011 and 2012, nymphs and adults were captured on mullein plants (*Verbascum thapsus* L.) found in Montréal (45.53°N; -73.59°W). In late November and December 2011, we collected apple (*Malus domestica* L.) tree cuttings from orchards of the Laurentians (45.51°N; -74.03°W) and from Eastern townships (45.26°N; -72.13°W), where high-densities of red spider mites have been observed previously. We stored these cuttings at a temperature of 1°C, in a refrigerated room. In February, cuttings were inserted into Styrofoam, put in acrylic glass cages with water, and placed in a growth chamber at 25°C, 60 % RH, and a 16:8 [L:D] photoperiod. Mullein bug nymphs hatched after 10 to 12 days and were manually collected with a paintbrush. Each nymph was then allowed to grow in a 10 cm diameter Petri dish, with cuts of mullein, potato and soybean leaves inserted in agar gelatine and *ad libitum* pollen, green peach aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (referred to as aphids thereafter), and two-spotted spider mites (referred to as spider mites thereafter). Captured adults were released into a cage to reproduce (see isogroup lines below).

Both aphids and mites came from laboratory breeding stocks. Aphids were reared on potato plants (*Solanum tuberosum*) (L.) and mites on soybean plants (*Glycine max*) (L.). Stocks were kept in a growth chamber at 25°C, 60 % RH, with a photoperiod of 16:8 [L:D] h. Aphids stocks had been maintained on potato plants for more than 5 years. Two-spotted mites, were obtained from Agriculture Canada (St-Jean-sur-Richelieu, Québec, Canada), and reared for one year before the beginning of the study.

2.3.2 Isogroup lines

Twelve isogroup lines of the mullein bug (referred to as line thereafter) were established from both insects captured in the field and coming from a captive stock maintained in our laboratory since 2007 (i.e. founders were captured in a commercial apple orchard at Oka, southern Québec). Each nymph was grown in a 10 cm Petri dish until it reached adulthood. To start a line, we released two virgin females and two males in a 30 X 30 cm acrylic glass cage each containing one mullein, one soybean, and two potato plants. Pollen, aphids, and mites were also provided *ad libitum*. Cages were kept in a growth chamber in the same conditions as above (except a additional 30 minutes pilot light to simulate twilight; copulation of mullein bug mainly occurs during twilight). Soybean and potato plants were replaced every seven to ten days, and pollen and preys were added weekly. Lines were allowed to reproduce for at least ten generations (assuming 40 days per generation, from egg to egg). Tests were run on individuals from the first to the tenth generation (G_1 to G_{10}). For each line tested individuals came from various generations (from two to eight different generations). Most lines were tested from the first or the second generation, whereas one line was tested from the fifth generation.

2.3.3 Zoophagy tests

Zoophagy in N3 to N5 nymphs and in adults mullein bugs was evaluated under laboratory conditions (25°C, 60 % RH, 16:8 [L:D] h). In the spider mite test, we used a 10 cm Petri dish that contains fresh mullein and soybean leaves in agar on which we added a cut of soybean leaf highly infected by mites. Mites were given 24 hours to move away from the fresh soybean leaf. Then, the old soybean leaf was removed from the Petri dish. Only highly infested (300 spider mites and more) soybean leaves were used to evaluate zoophagy. In the aphid test, we used a 10 cm Petri dish containing a cutting of fresh and uninfected mullein and potato leaves inserted in agar

gelatine on which 30 adult female aphids were cautiously transferred with a fine paintbrush. The aphid population usually grew during the 24-hour period of the test. Bugs (both nymphs and adults) were taken from their line cages and individually placed for 24 hours in a Petri dish containing a cut of fresh mullein, potato and soybean leaves inserted in agar gelatine to standardize the diet prior to the test. After that period, we transferred a bug into each of the Petri dishes using fine paintbrush, and allowed it to prey for 24 hours. At the end of that period, we counted the number of prey killed (either spider mites or aphids depending on the treatment).
We used between 14 and 44 bugs per line, for a total of 295 tested individuals on the spider mite diet (i.e. 27 N3 nymphs, 73 N4, 98 N5, and 97 adults), and between 15 and 35 bugs per line, for a total of 303 on the aphid diet (i.e. 48 N3, 75, N4, 85 N5 and 95 adults).

2.3.4 Nymphal development length

We measured nymphal development length from the beginning of N3 stage to adulthood. N1 and N2 nymphs were captured from their line cage and individually placed in 10 cm Petri dish for 24h to standardize diet prior to beginning the test (see above). Each nymph was then transferred into another dish containing a cut of fresh mullein, potato and soybean leafs inserted in agar gelatine. In the plant treatment, *ad libitum* quantity of pollen was added to the dish. The mixed-diet treatment consisted of *ad libitum* pollen, mites and aphids in the dish. Petri dishes were kept at 25°C, 70 % RH, and a photoperiod of 16:8 [L:D] h. Every two to three days nymphs were transferred into new dishes to ensure leaf freshness. Spider mites and aphids were added every two days to ensure nymphs on mixed diet always had animal resources.

We checked the development stage of each nymph every 24 hours until they reach adulthood or died. Nymphal development length was calculated as the number of days from the beginning of the 3rd nymphal instar stage to adulthood. Nymphal

development length was measured for 220 individuals, of which 167 reached adulthood (87 on a plant and 80 on a mixed diet). Between six and 18 individuals were recorded for each line, for a minimum of three individuals per diet and per line.

2.3.5 Statistical analyses

Genetic variance in zoophagy on either spider mites or aphids was estimated using mixed effect models. Number of spider mites or aphids killed over 24h was analysed as a function of bug development stage and sex (N3, N4, N5, adult males or females) and generation (centred on the mean) as fixed effects. We measured generation as the number of days since the foundation of the line divided by 40 (thus assuming a generation every 40 days). We included line ID as a random effect and ran four different models 1) included line ID as the only random effect (i.e. lines differ in their levels only), 2) and 3) an interaction between line ID and generation [i.e. 2) lines differ in their slopes only, and 3) lines differ in both their levels and their slopes, respectively], and 4) random slopes and correlation between levels and slopes. Using values of generation centred on the mean allowed us to estimate among-line variance could be estimated for in the middle of the experimental period. With the isogroup approach we expected some divergence among lines during the study period caused by genetic drift (see below). A significant interaction between generation and line ID would indicate that lines actually diverged from each other through time. We conducted model selection by retaining the model with the lowest AIC (Burnham and Anderson 2002; Bolker et al. 2009). With the isogroup-line approach, among-line variance in a trait could be used as an index of genetic variance for that trait (Hoffmann and Parsons 1988). Number of spider mites and aphids killed followed an overdispersed Poisson distribution. Because of the difficulty of estimating heritability (h^2) with negative binomial and quasi-Poisson distributions, we decided to run Generalized Linear Mixed Models (GLMM) fitting a Poisson distribution. All the

tests were run by using the function *lmer* from the package *lme4* (Bates et al. 2013) in the R package (R Core Team 2013).

We used random regression models (Dingemanse and Dochtermann 2012) to estimate genetic variance in reaction norms of nymphal development length on the two different diets. Individual values of development length were substracted by the minimum value for that trait (i.e. 9 days) and then square-root transformed to normalize the data. GLMMs on nymphal development length were run using a Gaussian distribution with diet and generation as fixed effects and line ID, diet and generation as a random effect. The random-effect structure was selected comparing the AIC of 11 models. As above, we selected the model with the lowest AIC value. Models with line ID and with the interaction between line ID and diet provides a way to test for the significance of genotype by environment interaction in zoophagy conditional to the type of prey. Significance of fixed effects were tested using a likelihood ratio test (LRT) at a threshold $a < 0.05$.

2.3.6 Heritability estimates

The isogroup-line approach provides an estimation of the narrow-sense heritability h^2 of the measured trait (Hoffmann and Parson 1988). Homozygosity within each line is assumed to increase with time because of genetic drift associated with small populations, thereby reducing the genetic differences among individuals within a line (Hoffmann and Parsons 1988). Lines were reared under similar laboratory conditions, and thus among-line genetic differentiation mostly account for the variation detected among lines.

Heritability was calculated from the adjusted intraclass correlation on the latent-scale (log-normal) following the equation described in Nakagawa and Schielzeth (2010):

$$RlnormA = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_\varepsilon^2 + ln(1/exp(\beta_0) + 1)}$$

Where σ_α^2 is the among-line variance, σ_ε^2 is the residual variance (equal to 1 in Poisson models), $ln(1/exp(\beta_0) + 1)$ represents the distribution-specific variance of a logit-link structure, and β_0 is the model intercept.

For nymphal development length as a function of diet and generation we calculated heritability as the ratio of among-line variance over the sum of among-line and residual variance following Nakagawa and Schielzeth (2010):

$$h^2 = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_\varepsilon^2}$$

2.3.7 Genetic correlation between zoophagy and nymphal development length.

We estimated genetic correlation between zoophagy of mullein bug nymphs (adults were discarded for this analysis) on spider mites and on aphids, using best linear unbiased prediction (BLUPs) from our GLMM models. We also correlated the lines random intercepts of both models on zoophagy (i.e. zoophagy on spider mites and aphids) with lines random intercept of the model on nymphal development length. BLUPs have been shown to provide highly reliable estimates of individual intercepts in a mixed model with one random effect (Martin and Pelletier 2011).

2.4 Results

2.4.1 Zoophagy on spider mites and aphids

On average, a mullein bug killed 24.94 ± 36.75 (mean ± s.d.) mites in 24 hours, for a maximum of 245 mites. Fourth instar nymphs (26.84 mites per day), fifth instar nymphs (35.16 mites per day), and adult males (22.45 mites per day) killed significantly more mites than adult females (13.60 mites per day; Tableau 2.1A). Number of mites killed by third instar nymphs (10.30 mites per day) was lower than that of adult females (Tableau 2.1A). Generation did not influence the number of mites killed in 24h (Tableau 2.1A). The best-fitted model on the number of mites

killed included lines as a random effect, the interaction between line ID and generation and a positive correlation between levels and slopes (0.78) (Tableau 2.2). The mean individual daily consumption in the different lines ranged from 11.13 to 42.75 mites (Figure 2.1). Among-line variance corresponded to a heritability (h^2) of zoophagy on mites of 0.54, after controlling for development stage and generation. The significant interaction between generation and line ID indicated that genetic drift was responsible for within-line changes across generations, whereas the positive correlation reveals that among-line differences in zoophagy increased with time.

Tableau 2.1. Effect of mullein bug developmental stages and generation on zoophagy (i.e. number of prey killed per day by one individual) on either two-spotter spider mites (A) and green peach aphids (B). Estimates represent the coefficient of regression from a generalized linear mixed-effect model (GLMM). Adult females are the reference in these analyses.

(A) Zoophagy on two-spotted spider mites

Fixed-effects	Estimates ± std-error	Z-value	P-value
Adult males	0.45 ± 0.05	8.79	< 0.0001
3rd instar nymphs	0.03 ± 0.08	0.46	0.65
4th instar nymphs	0.71 ± 0.05	15.69	< 0.0001
5th instar nymphs	0.98 ± 0.04	22.64	< 0.0001
Generation	0.27 ± 0.30	0.91	0.36

(B) Zoophagy on green peach aphids

Fixed-effects	Estimates ± std-error	Z-value	P-value
Adult males	-0.09 ± 0.11	-0.82	0.41
3rd instar nymphs	-1.06 ± 0.15	-7.22	< 0.0001
4th instar nymphs	-0.47 ± 0.10	-4.66	< 0.001
5th instar nymphs	-0.20 ± 0.09	-2.21	0.03
Generation	0.19 ± 0.08	2.29	0.02

Tableau 2.2. Akaike Information Criterion (AIC) for different generalized linear mixed-models on the number of two-spotted spider mites (295 individuals tested) or aphids (303 individuals tested) killed in 24 hours by mullein bugs (N3 to adults) from 12 isogroup lines. The fixed structure of the model included development stage and generation (centred on the mean). The selected model, based on the lowest AIC, is in bold.

Random effects	**AIC (spider mites)**	**AIC (aphids)**
+ (1 | line ID)	10034.67	902.21
+ (generation | line ID)	**9295.86**	884.06
+ (0 + generation | line ID)	10181.87	969.11
+ (1 | line ID) + (0 + generation | line ID)	9303.65	**882.86**

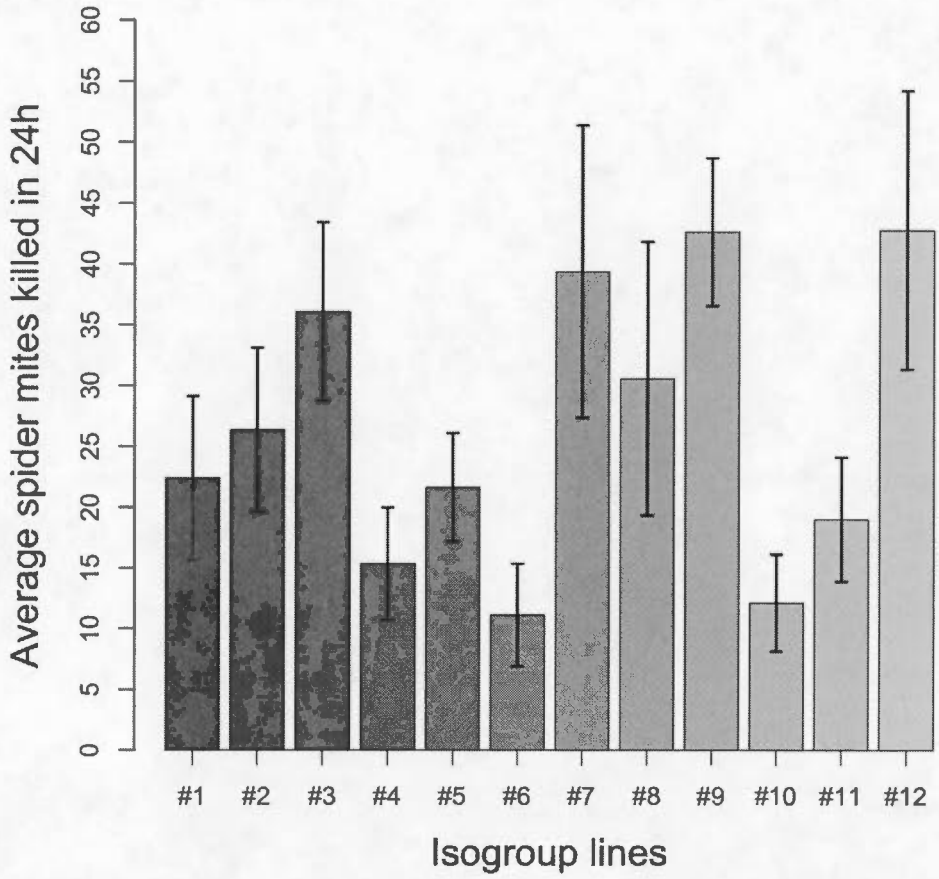

Figure 2.1. Number of two-spotted spider mites killed per day by 12 isogroups lines of nymphs (N3 to N5) and adult mullein bugs (295 individuals tested).

N3 to N5 nymphs and adults killed on average 3.15 (± 3.48 s.d.) aphids in 24 hours, for a maximum of 18 aphids. Third (1.33 aphids per day), 4th (2.81 aphids per day) and 5th (3.60 aphids per day) instar nymphs killed significantly less aphids than adult males (3.78 aphids per day) and females (4.00 aphids per day) (Tableau 2.1B). The number of aphids killed in 24h increased with generations (Tableau 2.1B). The best-fitted model included lines (random intercepts) and generation (random slopes), but

no correlation between them (Tableau 2.2). Lines killed on average between 0.84 and 5.38 aphids per individual per day (Figure 2.2). Heritability (h^2) of zoophagy on aphids was 0.16, after controlling for development stage and generation. The random slope generation indicated that genetic drift favoured divergence among lines, but it was not related to the level of zoophagy of lines.

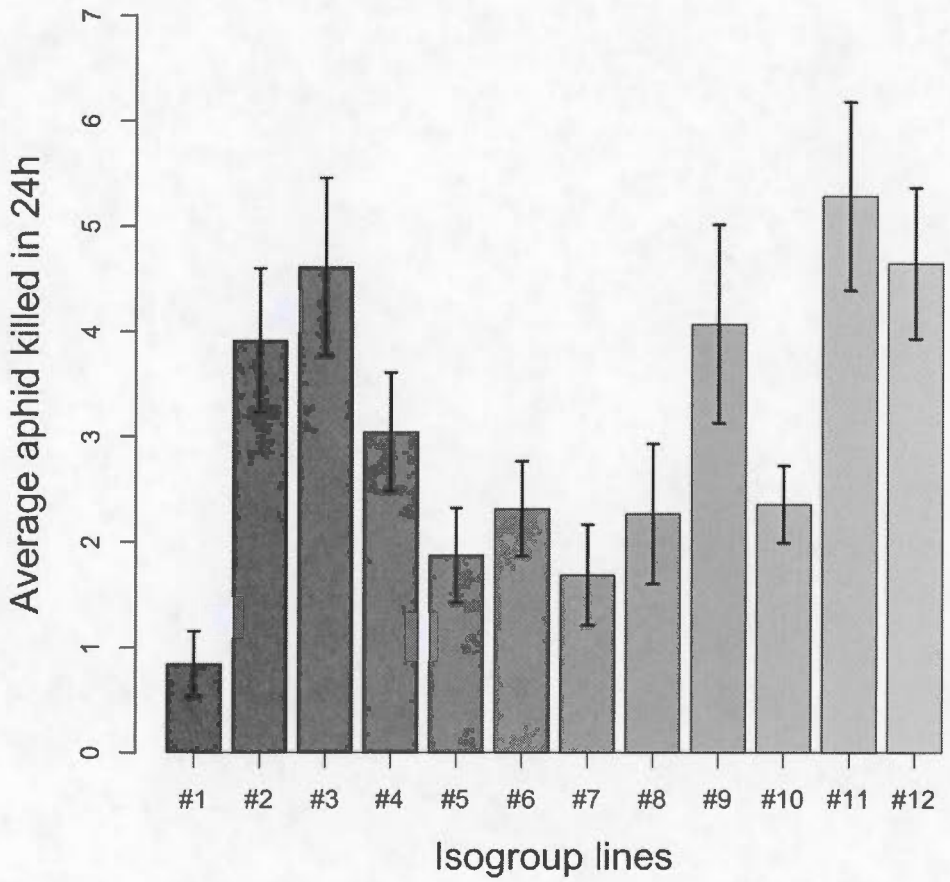

Figure 2.2. Number of green peach aphids killed per day by nymphs (N3 to N5) and adults of 12 isogroup lines mullein bugs (303 individuals tested).

2.4.2 Nymphal development length on plant and mixed diets

Third instar nymphs reached adulthood on average inÀàçç 11.76 (± 1.87 s.d.) days on a mixed diet and 12.47 (± 2.13 s.d.) on a plant diet. Development length did not differ significantly between the diets (LRT = 2.94, df = 1, p = 0.09) and among generations (LRT = 2.13, df = 1, p = 0.14). The best-fitted model on nymphal development included line as random effect, but no diet or generation (Figure 2.3; Tableau 2.3). Lines thus differed significantly in their nymphal development length ($h^2 = 0.12$), whatever the diet.

Tableau 2.3. Akaike Information Criterion (AIC) for different generalized linear mixed-model on mullein bug's nymphal development length (from N3 until adulthood) (167 individuals tested from 12 isogroup lines). The fixed structure of the model included generation (centred on the mean) and diet (mixed or plant). The selected model, based on the lowest AIC, is in bold.

Random effects	AIC
+ (1 | line ID)	**301.56**
+ (generation | line ID)	304.65
+ (0 + generation | line ID)	309.71
+ (1 | line ID) + (0 + generation | line ID)	303.22
+ (diet | line ID) + (0 + generation | line ID)	303.61
+ (0 + diet | line ID) + (0 + generation | line ID)	303.61
+ (1 | line ID) + (0 + diet | line ID) + (0 + generation | line ID)	305.61
+ (0 + generation + diet | line ID) + (1|line ID)	307.79
+ (generation + diet | line ID)	305.79
+ (0 + diet | line ID)	302.69
+ (diet | line ID)	302.69

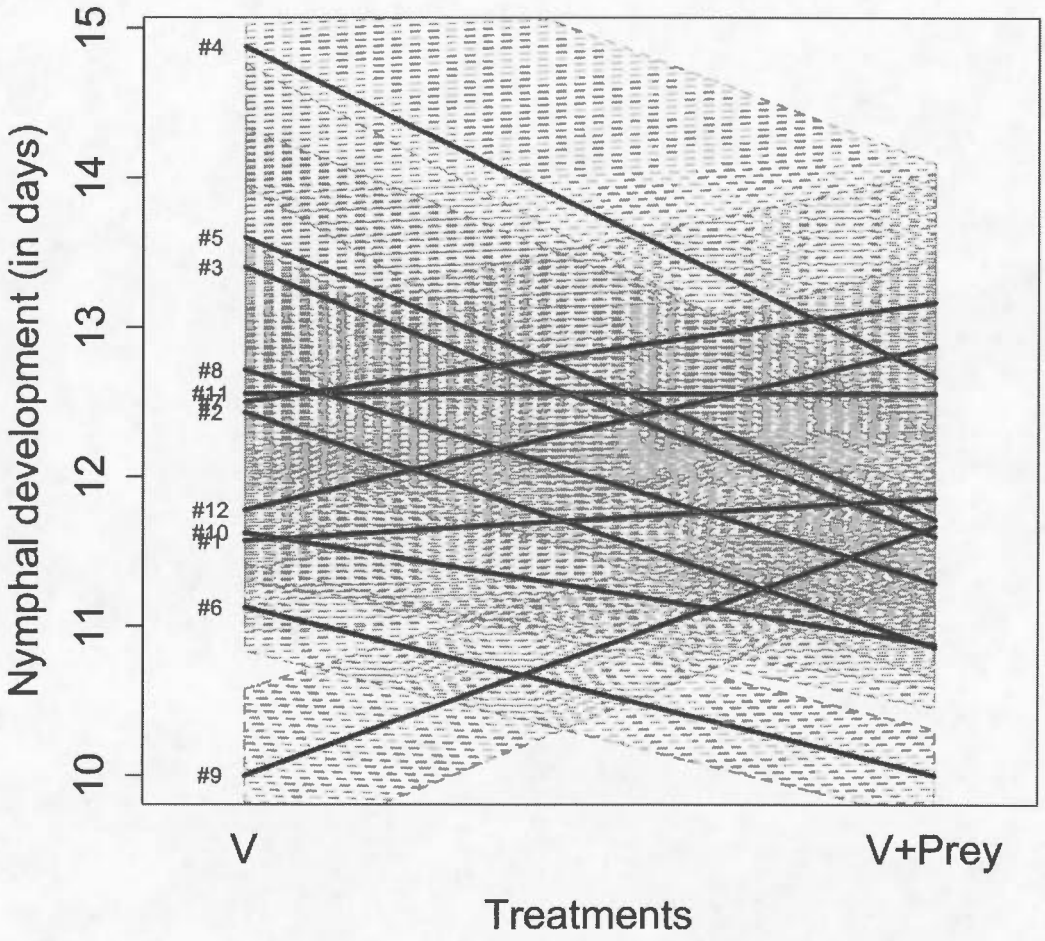

Figure 2.3. Nymphal development length (in days) of 3rd instar nymphs of mullein bug until adulthood under a mixed (i.e. prey and pollen) diet (V + prey) or a pure plant diet (V) for 12 isogroup lines under laboratory conditions (25°C, 70 % RH, 16:8 [L:D] h). Grey shading represents standard error for each isogroup lines.

2.4.3 Covariance between zoophagy and nymphal development length

The level of zoophagy on mites of each line was positively correlated with zoophagy on aphids (rho = 0.71; p = 0.01) (Figure 2.4). There was no correlation between nymphal development length and either zoophagy on mites (rho = 0.03; p = 0.92) or on aphids (rho = 0.21; p = 0.51).

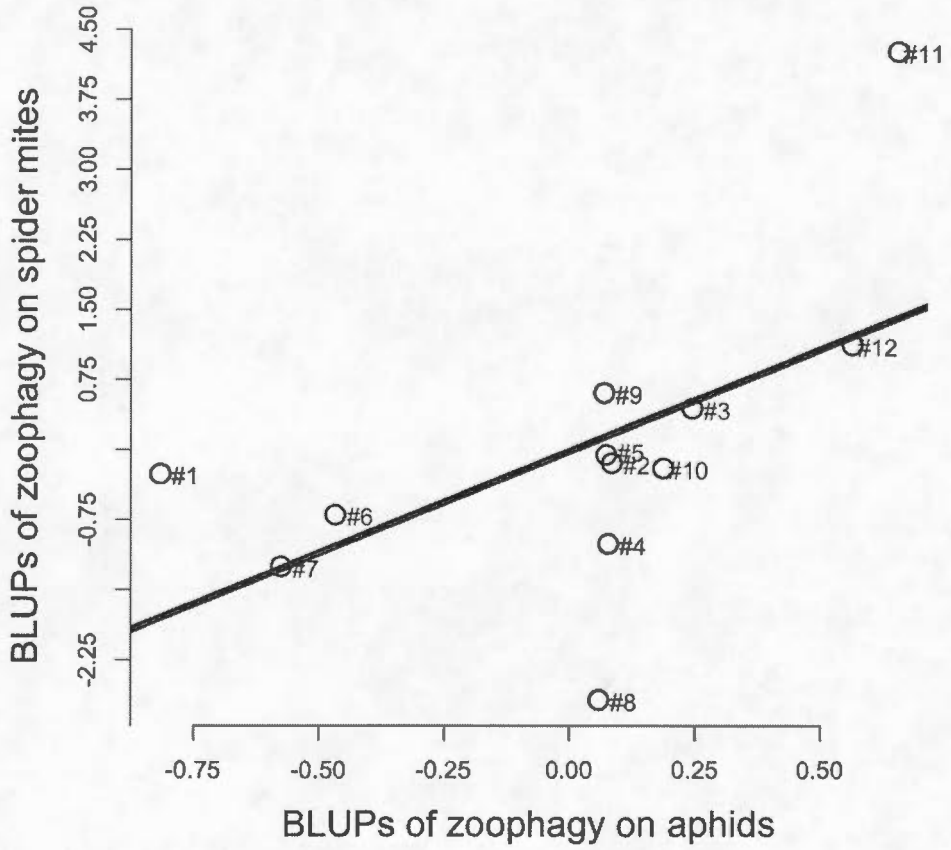

Figure 2.4. Correlation between isogroup-line zoophagy on two-spotted spider mites and on aphids under laboratory conditions. Level of zoophagy was estimated as the number of prey killed per bug, per 24h, and was controlled for nymphal stage and generation.

2.5 Discussion

Foraging behaviour is expected to vary among individuals within a population, because individuals have to cope with a broad range of resources that can vary in their quality, quantity, and distribution (Bolnick et al. 2003). Zoophytophagous mullein bugs showed genetic differences in zoophagy on both mites (= primary prey) and aphids (= secondary prey). Among-line variation in the level of zoophagy may be caused by differences in their ability to detect, catch and handle a particular prey or voraciousness. The positive genetic correlation between zoophagy on both preys indicates that overall, lines differed in their level of zoophagy, and not for a specific type of prey. Differences in zoophagy could also reflect differences in preference for and performance on animal *versus* plant resources. We observed genetic variation in nymphal development length, but no genotype by environment interaction in nymphal development length. Furthermore, we did not find any evidence for a genetic correlation between zoophagy and nymphal development length. This suggests that mullein bug nymphs can fully substitute animal resources by plant material. Therefore, highly zoophagous lines cope with the absence of prey by resuming foraging on protein rich flower pollen, an abundant and easy-to-acquire resource (in our experiment).

Mullein bugs have to deal with a high variability in resource availability, quality and type, both within and between generations. They produce two generations every summer in apple orchards in Canada (Thistlewood et al. 1990; Arnoldi et al. 1992). Individuals from the spring generation emerge early in the growing season, synchronized with both flowering apple trees and the emergence of red spider mites, and thus nymphs feed mainly on pollen, red spider mites, or small apples (Thistlewood et al. 1990; Arnoldi et al. 1992; Kain and Agnello 2013). Most of the adults then migrate to oviposit on an herbaceous host mainly the mullein *Verbascum thapsus* (L.) (Scrophulariaceae) and the sumac, *Rhus tiphina* (L.) (Anacardiaceae) (Thistlewood et al. 1990; Boivin et Stewart 1982). In contrast, when born in the

summer individual mullein bugs mainly rely on the leaves and pollen of these host plants (F. Dumont, personal observations). Some individuals, however, stay on apple trees during the summer. High spider mite densities, herbaceous hosts scarcity or risk related to migration may explain why mullein bugs stay on apple trees. In summer pollen and small fruits are no longer available on apple trees, and thus summer nymphs depend only on spider mites or aphids for their growth, as they do not feed on large apples (Boivin et al. 1982; O. Aubry, unpublished data). Thus, genetic variance in zoophagy may reflect the coexistence of lines with different degrees of zoophagy *vs* phytophagy. The maintenance of such variation may be caused by the heterogeneity of food resources in the environment. Adaptation to pollen-free environment (i.e. zoophagous strategy) may be advantageous when intraspecific competition is high (Bolnick et al. 2003), when mullein plants (or alternative herbaceous hosts) are scarce close to the orchard or when risks related to migration are high (e.g. if migration entail higher risk of predation).

High level of zoophagy and high voraciousness in some lines may also provide an adaptive advantage in environment with scarce food resources (Maupin and Riechert 2001). For example, desert spider *Agelenopsis aperta* (Gertsch) (Arachnida: Agelenidae) populations that inhabit sites of high prey availability are less responsive to prey than populations that occupy sites with low prey availability (Maupin and Riechert 2001). In a controlled laboratory environment, Maupin and Riechert (2001) found evidence for a genetic basis to population differentiation in responsiveness to prey. In zoophytophagous predators, encounter rate with prey is obviously very low compared to encounter rate with plant resources, and less predictable (Coll 1996; Coll and Izraylevich 1997). For instance, summer mullein bug nymphs that grow on mullein plants benefit from an extended period of flowering of their hosts and proximity with the abundant plant food resources. In contrast, summer nymphs growing on apple tree have their main food resources (i.e. spider mites) distributed heterogeneously, and such a distribution may explain the high responsiveness of some genotypes as these genotypes may have an advantage in this kind of habitat.

However, in our experiment we could not trace back the precise origin (i.e. mullein *vs* apple tree) of the line founders, and further work is needed to test whether mullein-hosted populations are characterised by lower levels of zoophagy than populations using apple tree as a host.

To our knowledge, our results are the first demonstration of genetic variation in foraging behaviour in a zoophytophagous bug. Several zoophytophagous species have important economical impact (Alomar and Wiedenmann 1996; Coll and Ruberson 1998; McGregor et al. 1999; Torres et al. 2010; Arnó et al. 2010), and it could be possible to take benefit of differences in foraging behaviour within the species to improve their efficiency as biological control agent. For example, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are released as biological control agent of white flies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and tomato borer *Tuta absoluta* (Meynick) (Lepidoptera: Gelechiidae) in tomato field and green house in Europe (Calvo et al. 2009; Luna et al. 2012; Nannini et al. 2012; Zappalà et al. 2013). According to our results, the efficiency of biological control releases of *M. pygmaeus* and *N. tenuis* may be improved by artificially selecting highly zoophagous genoptypes. Consequently, fewer bug individuals would be required to achieve the expected level of pest control. For instance, Nachappa et al. (2011) observed that high voracity lines of the predatory mites *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) at a ratio of 1:30 (predator: preys) achieved the same level of control on spider mites than unselected lines at a predator-prey ratio of 1:10. In the case of zoophytophagous predators, a decrease in the ratio of prey per predators improves their efficiency as biological control agent, but it is potentially associated with increased risks of damages on the plant itself (Sanchez 2008; Castañé et al. 2011). Thus, the used of fewer genetically improved individuals would mean lower risk of damage on crop by zoophytophagous predators. Moreover, we observed an increased level of zoophagy on aphids among generations, which may have been favoured by the high abundance of aphids in our rearing cages. This increase in zoophagy suggest

that rearing diet may have important consequences on effects zoophytophagous biocontrol agents would have in crop system. Consequently, the use of artificial diet (Uiterdijk et al. 1997; Vanderkerkhove and De Clercq 2010; Bonte and De Clercq 2010; Mollà et al. 2014) may lead to an increase of undesirable genotypes in the rearing population, and should be use with caution.

2.6 Acknowledgements

We gratefully thank Olivier Aubry for his precious help during rearing setting and discussion on this work. We thank Pascale Ayotte, Andréanne Lavallée, Laurent Fraser, Sheena-Émilie Boucher and Maïlys Allirand for their help on the capture of individuals, maintenance of isogroup lines and tests. F.D. was supported by a NSERC fellowship. The project expenses were financed by NSERC discovery grants program allowed to E.L and D.R.

CHAPITRE III

COEXISTENCE OF PHYTOZOOPHAGOUS AND ZOOPHYTOPHAGOUS STRATEGIES LINKED TO GENOTYPIC DIET SPECIALIZATION IN MULLEIN BUG *CAMPYLOMMA VERBASCI.*

Dumont, F., Lucas, E. & Réale, D.

3.1 Abstract

In agro-ecosystem, the benefits provided by zoophytophagous predators feeding on pest can be thwarted by damages caused when they feed on crop. In such predators, variation in feeding behaviour is observed both across and within populations. This may be caused by genetic variation in diet specialization, some genotypes specializing on plant resources whereas others relying mostly on prey to meet their energy and nutriment requirements. Genotypes within a zoophytophagous predator could vary tremendously in their ecological and economical impacts. Here, we tested the hypothesis that genotypes specialize either on prey or plant resources in the zoophytophagous mullein bug. In the laboratory, we offered the choice between two different diets to mullein bug nymphs from 11 different isogroup lines. The first diet was composed of two-spotted spider mites *Tetranychus urticae*, fresh soybean and mullein leave, and in the second we added *ad libitum* mixed flower pollen, a high-quality vegetal resource. We measured the occurrence of feeding either on prey or pollen and the proportion of time spent feeding on prey over total time spent feeding. Overall differences in zoophagy among isogroup lines remained consistent regardless of the presence or absence of pollen. Some lines switched from prey to pollen feeding when the pollen was available, revealing a negative genetic correlation between the

probabilities of feeding on both resources. A significant line by diet interaction in the proportion of time spent feeding on prey indicated the presence of a genetic basis for the plastic of diet preference in presence or absence of pollen. In absence of the preferred resource, zoophytophagous mullein bug nymphs act as generalists, but exhibited individual diet specialisation when facing the choice between high-quality animal and vegetal resources. Results suggest that genetic variation in feeding behaviour of zoophytophagous predations generates important differences among individuals in their level of benefits and damages caused in crop systems.

3.2 Introduction

The role of zoophytophagous predators (i.e. predators that can substitute prey for plant resources; Coll and Guershon 2002; Castañé et al. 2011) in agro-ecosystem can change as they switch from feeding on prey to feeding on plant resources (McGregor et al. 2000; Albajes et al. 2006; Castañé et al. 2011). Hence, a given zoophytophagous species can be either beneficial or considered as pest. Recently, we reported (Dumont et al. Chap. 2) genotypic variation in zoophagy of the zoophytophagous mullein bug *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) on two different types of prey: two-spotted spider mites *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) and green peach aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). These results indicated the existence of potential diet specialization and of different foraging strategies in that species: zoophytophagous individuals may prefer animal resources, whereas phytozoophagous individuals may consume essentially vegetal resources. Diet specialization in zoophytophagous predators could, thus, have considerable consequences on their role in agro-ecosystem as individuals may differ in associated benefits and damages to crop following their specialization on animal or plant resources.

The diversity of zoophytophagous and phytozoophagous strategies in a population results from gene by environment interaction (Svanbäck and Bolnick 2005). Genetic differences in morphology (e.g. size, body shape), physiology (e.g. digestive enzymes), or behaviour (e.g. voracity) among individuals modulate their potential to change their diet in response to variation in ecological factors such as resource abundance, intra- and interspecific competition, or risk of predation (Westoby 1978; Svanbäck and Bolnick 2005; Araújo et al. 2011; Dall et al. 2012). The ability of an individual to forage on different resources (e.g. prey versus plants) depends on the trade-offs between its ability to acquire, manipulate and digest each of these resources (Svanbäck and Bolnick 2005), and diet specialization should arise from such trade-offs (Bolnick et al. 2003). Therefore, diet specialization in zoophytophagous predators would generate variation among individuals or genotypes in the responses to the availability of different resources.

Diet specialization should be observed more frequently in more diverse environments (Bolnick et al. 2007, 2011). In presence of different resources, zoophytophagous genotypes should mostly feed on prey, whereas phytozoophagous genotypes should preferentially feed on plants. This situation should lead to higher genetic differences in mixed diet, revealing a high level of specialization in the population (Bolnick et al. 2007). In contrast, poorly diversified resources may lead to higher overlap in individual diet ranges. However, these hypotheses have yet to be tested in zoophytophagous predators.

In the present study, we tested the effect of plant resource (pollen) availability on the propensity to feed on animal resources (spider mites) of 11 isogroup lines of the zoophytophagous mullein bug. Our hypothesis was that diet specialization towards either a zoophytophagous or a phytozoophagous strategy modulates trophic switching from prey to plant resources in response to availability of pollen. We predict that: 1) propensity to feed on either prey or pollen varies among lines. In a previous study we

observed significant among-line differences in zoophagy in absence of pollen (Dumont et al. Chap. 2). Adding pollen as a potential resources to the bugs should increase these differences: when offered pollen phytozoophagous lines should switch their diet to pollen, but zoophytophagous lines should not change it. 2) When offered the choice between prey and pollen, individuals choose preferentially the resources that match their foraging strategy (i.e. animal-based diet or plant-based diet). Therefore, we expect that the availability of pollen generates differences in the proportion of prey versus pollen consumed between phytozoophagous and zoophytophagous lines.

3.3 Methodology

3.3.1 Mullein bug populations and isogroup lines

Mullein bug nymphs were reared in the biological control laboratory at Université du Québec à Montréal (Montreal, Quebec). Founder individuals originated from both apple trees and mullein plants collected in different regions of Quebec (i.e. Laurentians, Estrie, Monteregie, Quebec, Montreal). During 2011 and 2012 autumns, apple tree cuttings were collected in orchards and stored in a refrigerated room (1°C, 60 % relative humidity) to allow eggs to complete their diapause. In February, each cutting was placed in an acrylic glass cage at 25°C, 60 % RH and 16:8 [L:D] photoperiod until nymphs hatched (about 10 to 12 days). Nymphs were manually collected with a fine paintbrush and transferred in 10 cm diameter Petri dish on fresh cut of mullein, potato and soybean leaves inserted in agar gelatine to keep them moist. Mullein bug nymphs were supplied with green peach aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), two-spotted spider mites *Tetranychus urticae* (Koch) (Acarina: Tetranychidae) and pollen. We also used mullein bugs from laboratory rearing founded in 2007. Individuals of these rearing originated from

orchards of the Laurentians (Québec). Both green peach aphids and two-spotted spider mites came from stocks previously maintained in our laboratory.

Two females and two males mullein bugs were used to establish each isogroup line (referred to as line thereafter). We did not use only one pair for each line because of the high risks of extinction of the line under these conditions. Lines were held in 12 X 12 X 16 inch acrylic glass cage containing plant of mullein (x 1), soybean (x 1) and potatoes (x 2). Green peach aphids, two-spotted spider mites and pollen were provided *ad libitum*. Plants were replaced when necessary (about every 10 days for soybean and potatoes plants and 30 days for mullein plants). Individuals within lines were allowed to reproduce for about 16 generations. Foraging behaviour tests were run between the 2nd and the 15th generation, assuming a 40-day generation length (between 62 to 618 days after the foundation of lines). For each line, individuals of different generations were tested.

3.3.2 Tests on feeding behaviour

Feeding behaviour of 3rd and 5th instar mullein bug nymphs was observed in 10 cm Petri dish that contained cuts of fresh mullein and soybean leaves inserted into agar gel. In the without-pollen treatment, hundred adult and nymph two-spotted spider mites were manually transferred with a fine brush. In the treatment with pollen, we added 5 mg of pollen on the mullein leaf. Most spider mites remained on the soybean leaf where they foraged and laid their eggs. However, some individuals moved away from the soybean leaf to explore mullein leaf or agar gel.

Prior to the tests, some individual mullein bug nymphs from each line were put on a standardization diet (mullein leaf on agar gel) for 24 hours and kept in a rearing room at 24°C, 60 % RH and a [16L:8D] photoperiod. Developmental stage was noted and each nymph was gently deposited on a soybean leaf infested with two-spotted spider

mites in the test Petri dish. The behaviour of each nymph was observed under 10x binocular during 15 minutes. We voice-recorded the behaviour of the individuals and noted the time and the type of animal resources (i.e. all forms of two-spotted spider mites) and vegetal resources (i.e. soybean and mullein leaf, pollen) the nymph consumed. To feed, mullein bug nymphs bend their first and the second labial segments of the rostrum to penetrate food tissues, and pump up a mixture of saliva and diluted matter (Wheeler 2001). Exploratory probing (e.g. deeper penetration of the stylet) tends to be of short duration (Wheeler 2001). Thus, when the maxillary stylet is inserted in food tissues, the bug mainly flushes out content of the food resource. We thus considered that a nymph fed on a given resource when its maxillary stylet was inserted in the food item. From these observations, we measured the time spent consuming for each type of resource (i.e. animal and vegetal). We could thus estimate the proportion of time feeding on a given prey (i.e. spider mite adults, larvae or eggs) over the total time spent foraging on any available resources. We also recorded the occurrence of feeding on prey and on pollen as binomial data (feed or not during 15-minutes tests). We ran tests on 155 mullein bug nymphs for a total of 41,5 hours of observations (i.e. 5-12 individuals per line, per treatment). In 48 trials, nymphs did not consume any resource and the observations were discarded from the analysis; 107 tests remained in the analysis.

3.3.3. Statistical analysis

All analysis was implemented using R (R Core Team 2013) and *lmer* function of the *lme4* library.

We first tested for the effect of pollen on the probability of feeding on spider mite during a 15 min. trial (each nymph was scored 1 if observed feeding on prey and 0 if not; n = 107). Data followed a binomial distribution and we thus used a generalized linear mixed-effect model (GLMM) for binomial data. We included treatment (with

or without-pollen), developmental stage (N3 to N5) and generation (centred on the mean) as a fixed effect. The random structure was selected comparing 11 models that varied in their random effect structure involving line ID, treatment and generation (see Table 3.1 for details). The model with the lowest Akaike Information Criterion (AIC)) was selected as the best-fitted model (Burnham and Anderson 2002; Bolker et al. 2009).

We tested for the differences among lines in the probability of feeding on pollen (referred to as phytophagy thereafter), by running a GLMM for binomial data on the probability of feeding on pollen during a test. Developmental stage (N3 to N5) and generation (centered on the mean) were included as fixed effects. Four models were compared with or without line ID and the interaction between line ID and generation as random effects. In these models, data from observation of individuals without pollen were discarded (n = 56). We then estimated the genetic correlations between the probability of feeding on prey and on pollen using best linear unbiased predictors (BLUPs) from our GLMM models. Lines random intercepts of both models on probability of feeding feed on prey and on pollen were correlated.

We also analysed the proportion of time spent feeding on animal resources (referred to as zoophagy thereafter) over the total time spent feeding as a function of treatment (with or without-pollen), development stage and generation using two GLMMs for binomially distributed data (n = 107). We included line ID, treatment, generation and their interaction as random effects. The random structure was determined by the comparison of the AIC of 11 models that varied in random effects included (see Table 3.1).

For the three models, we used a likelihood ratio test (LRT) to estimate the statistical significance of fixed effects (using the function *drop1* in R).

Mullein bugs were tested at different generations. Some variation among individuals of the same line (i.e. within-line variance) resulted from genetic drift (Hoffmann and Parson 1988). Number of generations between the test and the foundation of the line (centred on each line's mean) was thus included as a covariate in the compared models. Model with and without the correlation among random effects were compared on the base of their AIC. If the best-fitted models included correlation, it indicates significant differences among lines in the change in behaviour with generation, whereas a correlation between intercepts and slopes provides information on how the lines differ in their behaviour with time.

We estimated the upper limit of narrow heritability in each models based on the isogroup lines repeatability of these behaviour (following Nakagawa and Schielzeth 2010). The following equations was used for binomial GLMM models:

$$\text{RlogitA} = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_\varepsilon^2 + \pi^{2/3}}$$

Where the residual variance σ_ε^2 is assumed to be 0 in normal binomial models and $\pi^{2/3}$ represents the distribution-specific variance of a logit-link structure. Heritability (h^2) on each treatment was measured independently when the random structure of the selected model included the variable treatment.

3.4 Results

In the without-pollen treatment 88.3 ± 32.5 % of the mullein bug nymphs (n = 51) fed on spider mites. In contrast only 50.0 ± 50.4 % of bugs fed on spider mites in the pollen treatment tests (n = 51). This difference was statistically significant (LRT = 13.25, df = 1, $p < 0.0003$). Development stage did not have any effect on zoophagy (LRT = 0.007, df = 2, p = 0.99), nor did the generation (LRT = 0.90, df = 1, p = 0.34). The selected model included only line ID as random effect: lines differed

significantly in their zoophagy (Figure 3.1, Tableau 3.1). Heritability (h^2) of zoophagy (in both treatments) was estimated to be 0.28. Lines did not show any significant differences in their reaction norms in response to pollen availability, and zoophagy did not change across generations.

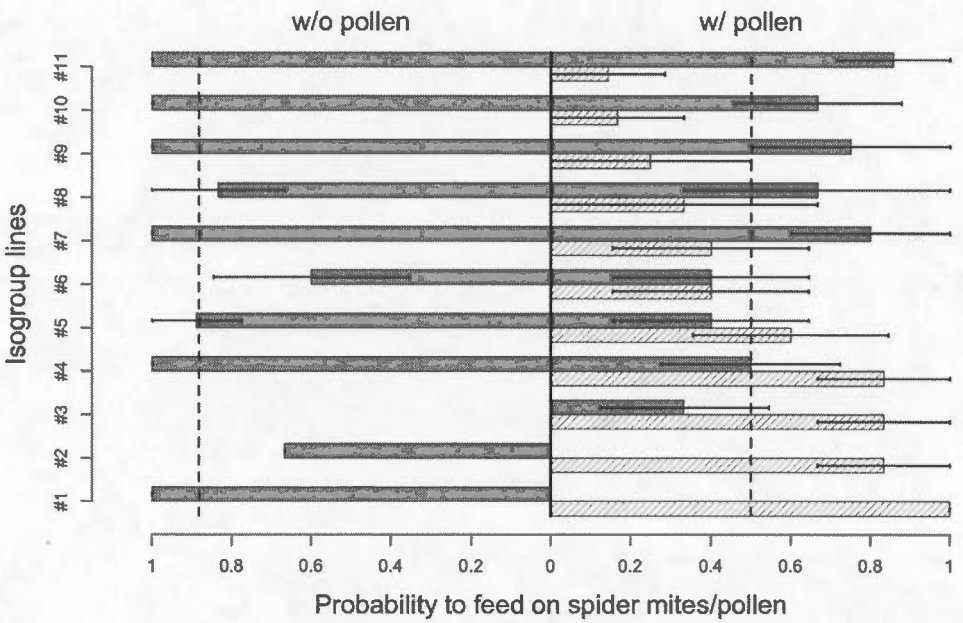

Figure 3.1. Probability of feeding on spider mites (dark bars) or pollen (light bars) for nymphs of 11 mullein bug isogroup lines, during a 15-minutes test, in two treatments without (left side) or with pollen (right side). Dashed grey bars represent population's mean probability of feeding on spider mites in both treatments.

Tableau 3.1. Akaike Information Criterion (AIC) for different generalized linear mixed-model on mullein bug's probability to feed on prey and to feed on pollen, and the proportion of animal resources in their diet (107 individuals tested from 11 isogroup lines). The fixed structure of the model included generation (centred on the mean of each line), development stage and treatment (with or without pollen). Selected models, based on the lowest AIC, are in bold.

Random effects	AIC (feed on prey)	AIC (feed on pollen)	AIC (proportion of animal resources)
+ (1 | line ID)	**121.13**	**81.63**	9591.17
+ (generation | line ID)	124.80	85.53	6911.55
+ (0 + generation | line ID)	125.06	82.91	8937.37
+ (1 | line ID) + (0 + generation | line ID)	123.13	83.63	6915.97
+ (diet | line ID) + (0 + generation | line ID)	126.96	n/a	5215.03
+ (0 + diet | line ID) + (0 + generation | line ID)	126.96	n/a	5215.03
+ (1 | line ID) + (0 + diet | line ID) + (0 + generation | line ID)	129.13	n/a	5217.03
(0 + generation + diet | line ID) + (1|line ID)	133.0	n/a	5202.75
+ (generation + diet | line ID)	129.81	n/a	**5200.75**
+ (0 + diet | line ID)	124.96	n/a	7344.42
+ (diet | line ID)	124.96	n/a	7344.42

In the pollen treatment 51.8 ± 50.4 % of mullein bug nymphs fed on pollen. Neither developmental stages (LRT = 3.04, df = 2, p = 0.22) nor generation had significant effect on phytophagy (LRT = 0.01, df = 1, p = 0.91). The best-fitted model included line ID as random effect, indicating that lines showed a difference in phytophagy

(Figure 3.1). Estimate of heritability (h^2) of phytophagy was 0.20, after controlling for development stage and generation. Number of generations between the test and the foundation of the line had no significant effect on phytophagy.

Isogroup-line zoophagy was significantly and negatively correlated with phytophagy (rho = -0.85; p = 0.002) (Figure 3.2).

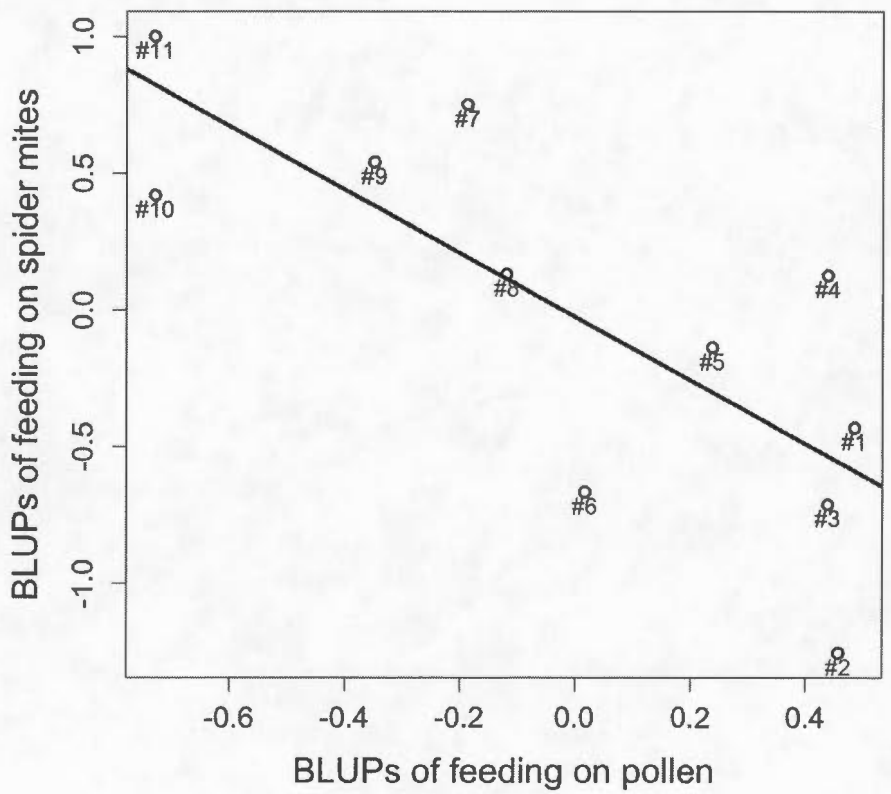

Figure 3.2. Correlation between the probability of feeding on spider mites and the probability of feeding on pollen for 11 mullein bug isogroup lines.

In the without-pollen treatment, bugs spent 86.2 ± 33.5 % of their total feeding time (all resources included) eating animal resources (i.e. two-spotted spider mite adults, nymph and eggs; Figure 3.3). This proportion was 46.4 ± 48.2 % when nymphs had access to pollen, which was not statistically significant (LRT = 2.77, df = 1, p = 0.10). The addition of pollen was associated with a decrease in time spent feeding on two-spotted spider mites and on mullein leaves (Tableau 3.2). The N3 to N5 nymphal stages varied in the proportion of time they devoted to animal resources over time spent feeding on all the resources (LRT = 14.02, df = 2, p = 0.0009). There was no effect of generation on the proportion of time nymph spend feeding on animal resources (LRT = 0.006, df = 1, p = 0.94). The model with the lowest AIC included line ID as random intercept and both generation and treatment as random slopes, as well as the correlation among these parameters (Tableau 3.1). Lines varied significantly in the proportion of time they spent feeding on animal resources, and in their reaction norm in response to pollen availability (Figure 3.3). We found a heritability of 0.16 for the proportion of time spent feeding on prey when pollen was not available and of 0.68 when pollen was available. The negative correlation (r = -0.91) between the proportion of time spent feeding on prey in either treatment with or without pollen indicates that lines which mainly fed on pollen switched to prey when pollen was not available. Number of generations between the test and the foundation of the line had a significant effect on time spent feeding on prey. Variation in the proportion of animal resources across generations varied among lines (r = -0.25), indicating that genetic drift operates changes within lines but did not increased differences among lines.

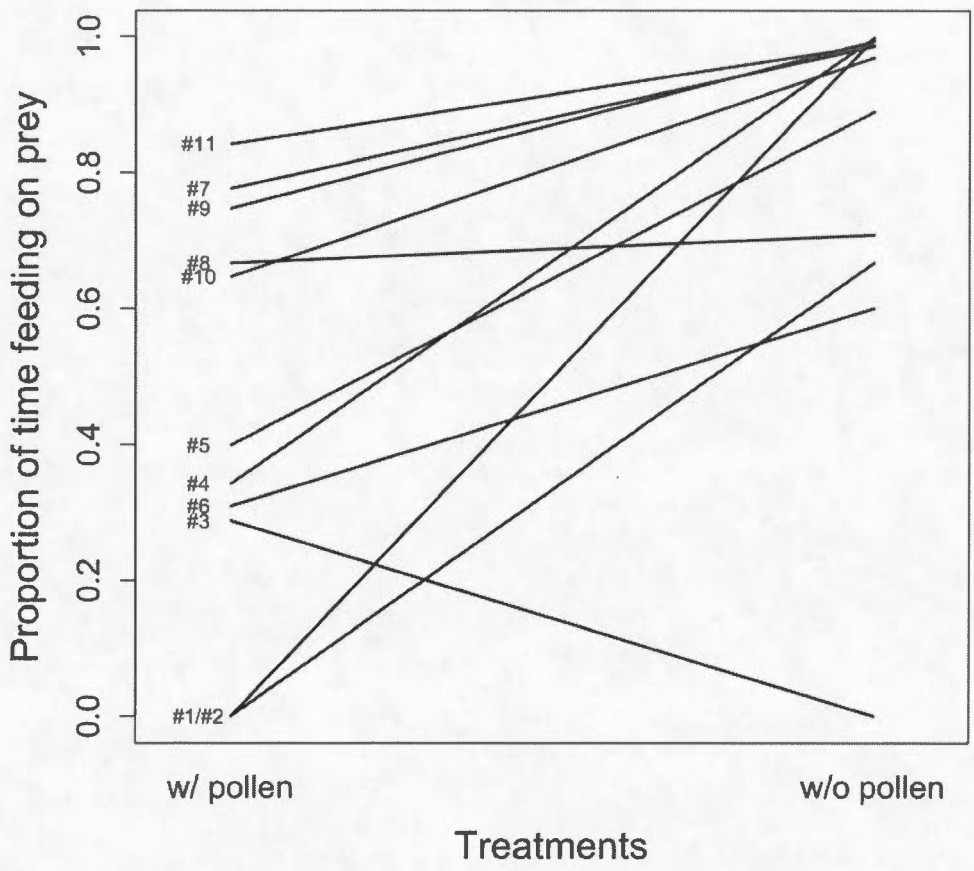

Figure 3.3. Effect of pollen availability on the proportion of time spent feeding on prey over the total time spent feeding, for nymphs of 11 isogroup lines of mullein bug, during a 15 min. test. Numbers refer to isogroup lines in Fig. 3.1.

Tableau 3.2. Time spent feeding on the different food items in the without-pollen and pollen treatments for nymphs of 11 isogroup lines of mullein bug, over a 15 min test. Number of individuals in the without-pollen treatment: 51; Number of individuals in the pollen treatment: 56.

Time spent feeding (s)	Without pollen Average ± SD	With pollen Average ± SD
Total	471.90 ± 206.64	376.89 ± 254.19
Two-spotted spider mites	438.7 ± 241.6	213.1 ± 273.2
Pollen	N.A.	156.1 ± 224.5
Mullein leaves	26.7 ± 102.1	0.2 ± 1.3
Soybean leaves	6.43 ± 18.0	7.5 ± 28.9
Agar gel	2.2 ± 12.4	2.6 ± 17.6

3.5 Discussion

Diet specialization results from the fact that individuals often exploit different subsets of the resources available to the population (Bolnick et al. 2003). Such specialization can be caused by genetic variation in the ability to detect, capture, handle or digest different resources or in the response to changes in ecological conditions (Araújo et al. 2011). In zoophytophagous predators, diet specialization would be defined by among-individual differences in the proportions of animal and plant resources consumed and in changes in diet in response to changes in the relative abundance of each type of resource. Our results show that in the zoophytophagous mullein bug individuals did not behave as generalists eating any type of resources as a proportion of their relative abundance, but there are genetic differences in the relative proportion of different types of resources eaten. In our study, we confirmed the hypothesis that trophic switching (from prey to plant resources) depends on diet specialization

towards either a zoophytophagous or a phytozoophagous strategy. We show that depending on their diet specialization individual mullein bugs differed in how they modulate their diet in response to the relative availability of pollen: phytozoophagous lines adjust their time spent feeding on prey to a higher extend than zoophytophagous lines. Furthermore, lines varied in their zoophagy regardless of whether or not they had access to pollen. Among-line differences in the level of zoophagy increased when pollen was provided as an additional resource. Interestingly, diet specialization is detected at the genetic level with an increase in heritability of diet choice in presence of pollen. Lines that fed mainly on pollen switched to a diet with a high proportion of animal resources, in the absence of pollen; revealing that pollen was not surrogated by others plant material. Thus, mullein bugs foraging behaviour would rely both on their diet specialisation and the availability of spider mites and pollen as alternative resources.

When one of the main resources is absent (i.e. pollen) the differences between zoophytophagous and phytozoophagous strategies were reduced. In our tests, the absence of pollen led to an increase in predation on spider mites, and as heritability of diet specialization decreased, individuals behaved more as generalists. In the case of zoophytophagous predators, the slight differences in voracity on a single resource among individuals would increase considerably when individuals have the choice between several resources. The genetic variation in the response to the availability of pollen we observed in mullein bug strongly supports this hypothesis. Consequently, the genetic diversity and the relative frequency of each type of foraging strategy within population of zoophytophagous predators would be significant in the ecological and economical role that these insects play in agro-ecosystem.

Zoophytophagous predators vary in their status depending on the region. For instance, the mullein bug is considered as a pest in apple and peer orchards in British Columbia, Nova Scotia, and New York State, but is reported as potentially beneficial

in Quebec, or England (McMullen and Jong 1970; Boivin and Stewart 1982). Consequently, some authors referred to mullein bug as a zoophytophagous predator (Kinkorova and Kocourek 2000; Reding et al. 2001; Fréchette et al. 2008; Aubry et al. 2011), whereas others have qualified it as being phytozoophagous (Sanford 1964; Torres et al. 1999). Our results showing genetically-based individual diet specialization are consistent with the hypothesis that mullein bug's populations can vary in their relative proportion of zoophytophagous *vs* phytozoophagous genotypes. As a consequence one may wonder what particular type of ecological or anthropic conditions drives the overall differentiation in the diet of different mullein bug's populations. Pesticides can affect zoophytophagous predators directly by lowering their survival and reproduction, including plant-incorporated pest resistance (Moser and Obrycki 2009; Torres et al. 2010; Arnó and Gabarra 2011), or indirectly by decreasing prey abundance (Kinkorova and Kocourek 2000). Acaricides or systemic insecticides may affect zoophytophagous genotypes to a higher extent than phytozoophagous ones. First generation female mullein bugs lay their eggs either on herbaceous plants (e.g. mullein plants *Verbascum* spp.) or apple tree (McMullen and Jong 1970). Nymphs emerging on apple tree during mid-summer (i.e. July) could be affected by chemical treatments used to control pests in orchards to a higher extent than nymphs emerging on herbaceous plants. Zoophytophagous lines may choose apple tree hosts because spider mites may be abundant during summer (but pollen is not available). Considering the relatively high heritability of the response to pollen availability ($h^2 = 0.68$), such a process could rapidly become undesirable, as strong unintentional selection pressures favouring phytozoophagous genotypes may permanently increase the cost of sheltering more phytozoophagous bugs in the agro-ecosystem.

Genetic differences are usually ignored in most biological control studies, and the general practice is to assume ecologically equivalent and interchangeable individuals. However, ecological processes such as predator-prey interactions and dynamics

depend on individual-level ecological interactions that can substantially vary following inter-individual variation (Bolnick et al. 2003; Hughes et al. 2008; Bolnick et al. 2011; Dall et al. 2012). Nachappa et al. (2011) observed that genetic variation in prey consumption, conversion efficiency and dispersal in predatory mites *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) affect predator-prey interactions, long-term population dynamics as well as efficiency in the biological control of two-spotted spider mites. In the zoophytophagous mullein bug, differences in the zoophagy between lines might generate various levels of benefits in agro-ecosystems. The individual-based and genotypic-based approaches bring us further in the understanding of the ecological and economical roles of omnivorous predators. Our results on mullein bugs could be extended to other zoophytophagous mirids used in agronomy. Several zoophytophagous mirids such as *Macrolophus pygmaeus* (Rambur) (previously *M. caliginosus*), *Nesiodiocoris tenuis* (Reuter), *Dicyphus tamaninii* (Wagner), and *Dicyphus hesperus* (Knight) (Hemiptera: Miridae), are efficient predators of white flies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and tomato borer *Tuta absoluta* (Meynick) (Lepidoptera: Gelechiidae) in tomato field and greenhouses (McGregor et al. 1999; Calvo et al. 2009; Luna et al. 2012; Nannini et al. 2012; Zappalà et al. 2013). All these predators can damage crops by puncturing fruits and/or vegetative parts (Castañé et al. 2011). Just as the status of mullein bugs can be completely different between populations, the status of several zoophytophagous mirids in tomato crops is highly controversial as their efficiency and propensity to damage plants and more specifically fruits, varied considerably from a study to another (Arnó et al. 2010). The study of genetic variations underlying foraging behaviour in mirids could then give further understanding of population's variation in feeding habits in this ecologically and economically important heteropteran family.

3.6 Acknowledgements

Thanks to Pascale Ayotte, Andréanne Lavallée, Laurent Fraser, Sheena-Émilie Boucher and Maïlys Allirand for their help in maintenance of isogroup lines and tests. We are grateful to Olivier Aubry for help during rearing setting and discussion on this work. F.D. was supported by a NSERC fellowship. The project expenses were financed by NSERC discovery grants program allowed to E.L and D.R.

CHAPITRE IV

EFFECT OF ALTERNATIVE PREY OR PLANT RESOURCES ON CANNIBALISM BY PREY- AND POLLEN-SPECIALIZED ISOGROUP LINES OF THE MULLEIN BUG *CAMPYLOMMA VERBASCI*.

Dumont, F. Réale, D. & Lucas, E.

4.1 Abstract

Zoophytophagous predators, which can feed on different prey and plant resources, can damage agricultural crops when their number is high relative to that of their prey. Cannibalism has been observed in many arthropod species. Cannibalism may increase predator survivorship under low prey density. From an applied point of view, cannibalism in zoophytophagous species can reduce predator density, and potentially divert individuals from feeding on plants, thereby reducing plant damages. An individual's tendency for cannibalism may depend on its genotype in link with its specialization for prey or plant resources, in interaction with the availability of alternative food resources. We hypotheses that 1) line specialized for prey consumption show higher level of cannibalism than pollen-specialized line, and 2) both lines decrease cannibalism in presence of their preferred resource. We tested these hypotheses with two isogroup lines of the zoophytophagous mullein bug *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) either specialized on animal (mites) or on plant (pollen) diets. Under laboratory conditions, we allowed large 4^{th} and 5^{th} nymphal instars to feed on small 1^{st} and 2^{nd} instar during 24 hours, in absence of additional resources, and in presence of either two spotted spider mites, or pollen. Cannibalism was significantly reduced by the availability of either prey or pollen, but

prey had a lower effect than pollen. According to our 1st hypothesis, the intensity of cannibalism was always higher in the prey-specialized line than in the pollen-specialized line regardless the availability of supplemented resources. The pollen-specialized line decrease cannibalism only when pollen was available. Those results indicate that cannibalism is potentially a regulating force in prey-specialized line, but not in pollen-specialized line.

4.2 Introduction

Cannibalism (i.e. intraspecific predation) is common among natural populations of insects and others arthropods (Fox 1975; Polis 1981). In her review, Fox (1975) reported that cannibalism is frequent in omnivorous predators, which can feed on several alternative food resources. Cannibalism results from opportunistic predation on vulnerable individuals or stages, and should increase with population density. Feeding on conspecifics may allow predatory organisms to complete their development under low quality or quantity resources conditions (Ullyett 1950; Snyder et al. 2000; Mayntz and Toft 2006). Cannibalism may, therefore, allow those organisms to persist in crop agro-ecosystem until their prey populations increase. Moreover, cannibalism by larger individuals preying on smaller ones can reduce population density and the presence of younger stages in the population (Claessen et al. 2004; Rudolf 2007; Torres et al. 2010). In zoophytophagous predators that can either be beneficial when feeding on prey, or harmful when feeding on plant materials (Castañé et al. 2011) cannibalism may modulate the risks of damages to crops. Damages caused on crop by zoophytophagous species usually increase when their population is high relative to their prey populations (Sanchez 2008). Thus, cannibalism should have the beneficial effect of decreasing population density of zoophytophagous predators in crop systems when pest are less abundant (Rudolf 2007).

Resources availability can modulate cannibalism in zoophytophagous predators (Leon-Beck et al. 2007; prey: Wagner and Wise 1996, 1997; Hironori and Katsuhiro 1997; plant: Cottrell and Yeargan 1998). Leon-Beck et al. (2007) observed that increased prey and pollen availability decreased equally cannibalism in the zoophytophagous minute pirate bug *Orius laevigatus* (Say) (Hemiptera: Anthocoridae). However, individuals or genotypes specialized on particular types of resource may show different levels of cannibalism as a result of changes in food resource. In previous studies (Dumont et al. Chap. 2, 3) we demonstrated the presence of genetic specialization for prey or for pollen diets in the mullein bug *Campylomma verbasci* (Meyer) (Hemiptera: Miridae). In presence of pollen, pollen-specialized genotypes switched from prey to pollen, whereas more zoophagous genotypes kept feeding on prey. Nymphs of mullein bug provide benefits in agro-ecosystem when feeding on spider mites or aphids (Arnoldi et al. 1992), but can be harmful when feeding on apple fruitlets (Boivin and Stewart 1982; Kain and Agnello 2013). In this study we use two isogroup lines contrasted in their level of zoophagy to assess whether genetic differences in diet specialisation is linked to the intensity of cannibalism. We hypothesise that: 1) the isogroup line specialized on prey shows higher level of cannibalism than the pollen-specialized line; and 2) both lines would increase cannibalism in response to the decline in their specialized resource.

4.3 Methods

4.3.1 Populations and rearing conditions

In previous studies, we determined the level of zoophagy and diet specialisation in 12 isogroup lines (Dumont et al. Chap. 2, 3). Here we used the two isogroup lines that exhibited contrasted levels of zoophagy on spider mites. Both lines were founded from two virgin females and two males either captured as nymphs in the field (i.e. apple orchards and mullein plants) or from a rearing populations. Individuals within

isogroup lines were allowed to reproduce for 15 generations (assuming a generation every 40 days). Details on the foundation and breeding of lines can be found in Dumont et al. (Chap. 2, 3). N1 and N2 nymphs were randomly picked up from our stock population that was founded from individuals captured in apple orchards and on mullein plants in different regions of Quebec (Canada), in 2011 and 2012. We used young nymphs from the stock population to avoid uncontrolled interference of prey defensive behaviour on the rate of cannibalism of each of the two lines. N4 and N5 nymphs (potential canibals) came from the two isogroup lines: 1) the prey-specialized line, and 2) the pollen-specialized line.

4.3.2 Cannibalism tests

To evaluate the rate of cannibalism, we put together four young nymphs (N1 or N2; the expected prey) and two older nymphs (N4 or N5 the potential cannibals) in a Petri dish for 24 hours.

Prior to the beginning of the tests, the older nymphs were individually placed for 24 hours in a 10 cm diameter Petri dish containing cutting of mullein leaf in agar gel. Cannibalism tests were run in 5 cm diameter Petri dishes containing a fresh cut of bean leaf embedded upside down in agar gel. All Petri dish had a hole in the lid covered with fine muslin for humidity control. The bean leaf covered all the space in the Petri dish, thus, providing a surface of 19.64 cm^2 to the nymphs. Three treatments were provided: 1) No resource treatment: without additional resource; 2) Pollen treatment: with a small quantity of pollen deposited on the middle of the Petri dish; 3) Prey treatment: a 1 cm^2 cutting of bean leaf well-infested with two-spotted spider mites deposited in the middle of the Petri dish.

Just before the beginning of the test, four young nymphs (N1 and/or N2) were gently introduced in the Petri dish with a small paintbrush. Two older nymphs (N4 and/or N5) were then introduced in the Petri dish. The Petri dishes were closed with

Parafilm and placed into a growth chamber at 25°C, 70 % R.H., and 16:8 h L:D for 24 hours. The number of nymphs killed was counted after this period. Mullein bugs consume their prey and empty their carcases, and thus cannibalism on mullein bug can easily be differentiated from natural death. Each line was tested 20 times in each of the three treatments for a total of 120 individuals tested (n = 120).

4.3.3 Statistical analysis

First, a generalized linear model (GLM) for Poisson distribution was implemented to test the effect of treatment (control (no added food), prey (two-spotted spider mites) and pollen), isogroup line ID (prey or pollen-specialized line) and two-way interactions between these factors. Variables were selected using a backward stepwise and only variables with a p-value under 0.05 were kept in the models. The p-value of each variable was obtained using *drop1* function for Poisson GLM, which drops each explanatory variable in turn and compares differences among models to a Chi-square distribution (Zuur et al. 2009). An all-pairwise comparisons of Tukey was implemented using *glht* function (package *multcomp*; Hothorn et al. 2008) was used to detect differences among all three treatments (Crawley 2007; Herberich et al. 2010). Then, two GLM models for Poisson distributed response variable were run to test the effect of treatment on cannibalism by each line taken separately. All analysis was performed using R (R Core Team 2013).

4.4 Results

Mullein bugs killed 1.10 ± 1.22 (mean ± s.d.) nymphs per day in the control treatment (no food added), 0.76 ± 0.81 in the Prey treatment, and 0.28 ± 0.45 in the Pollen treatment, respectively (Figure 4.1). Differences among all treatments were statistically significant (LRT = 21.52; $p < 0.0001$), indicating that both prey and pollen treatments reduced cannibalism (Tableau 4.1).

Tableau 4.1. All-pairwise comparisons of Tukey for Poisson GLM comparing treatments (control (no added food), prey (two-spotted spider mites), and pollen) in tests of cannibalism in mullein bug nymphs.

Comparisons	Estimates ± s.e.	z-value	p-value
Prey – control	-0.61 ± 0.25	-2.39	0.04
Pollen - control	-1.39 ± 0.34	-4.11	< 0.001
Prey - pollen	0.78 ± 0.36	2.14	0.08

Rate of cannibalism was significantly higher in the prey-specialized line (0.85 ± 1.09 nymphs /day) than in the pollen-specialized line (0.47 ± 0.72 nymphs/d), regardless of the treatment provided (LRT = 6.79, p = 0.009) (Figure 4.1).

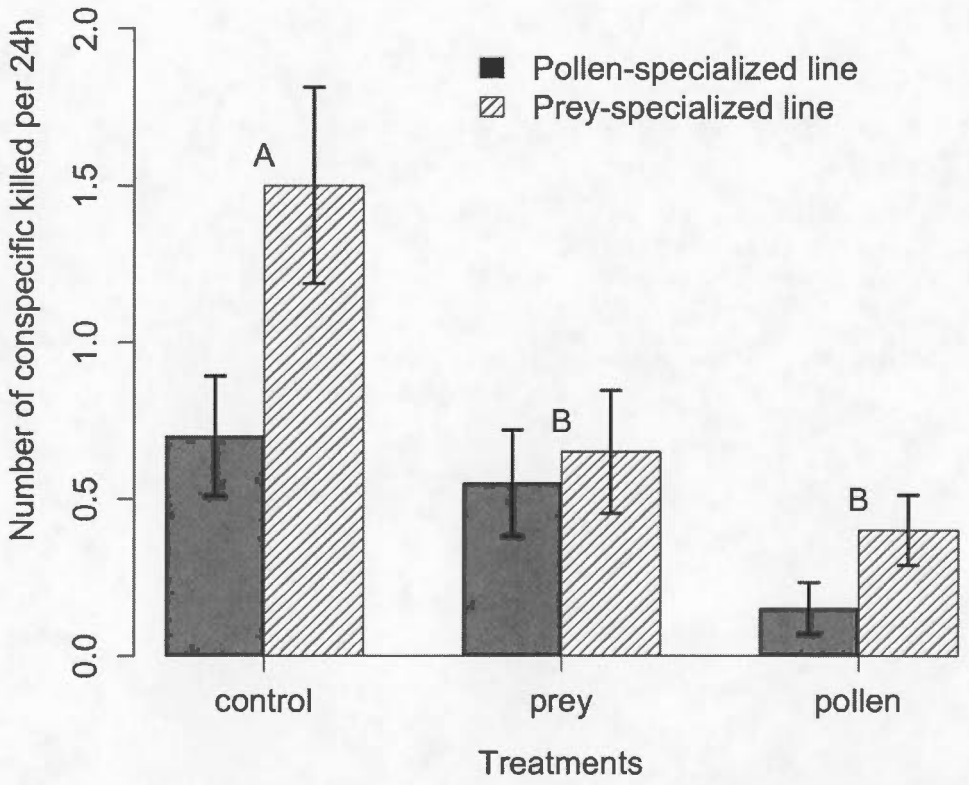

Figure 4.1. Number of conspecifics killed per 24 hour by mullein bugs that were feed on diet without additional food (control), with two-spotted spider mites or with pollen in prey- and pollen-specialized lines. Letters referred to differences among treatments.

The two-way interaction between treatment and line was not statistically significant and thus was removed from the model (LRT = 1.68, p = 0.43). However, the availability of both prey and pollen decreased significantly cannibalism in the prey-

specialized line (Tableau 4.2), whereas only pollen effectively decreased cannibalism in the pollen-specialized line (Tableau 4.2).

Tableau 4.2. The effect of two-spotted spider mites and pollen availability on the rate of cannibalism in Prey-specialized line and Pollen-specialized line of mullein bug.

Treatment	% reduction	z-value	p-value
Prey-specialized line			
pollen	73.3 %	-3.32	0.0009
prey	57.7 %	-2.52	0.01
Pollen-specialized line			
pollen	78.6 %	-2.42	0.02
prey	21.4 %	-0.60	0.55

4.5 Discussion

Cannibalism can be an important source of mortality both under laboratory and field conditions (Fox 1975; Polis 1981; Wagner and Wise 1996). Furthermore, it can affect biological control by its effect on population dynamics (Hironori and Katsuhiro 1997; Claessen et al. 2004; Wise 2006). In zoophytophagous predators, availability of alternative prey or plant resources is assumed to reduce the intensity of cannibalism (Leon-Beck et al. 2007). However, diet specialisation can modulate how individuals or genotypes interact with alternative food resources (Bolnick et al. 2003; Svanbäck and Bolnick 2005; Dumont et al. Chap. 2), including conspecifics (Pruitt and Riechert 2012).

Our study shows that mullein bug's lines that differ in their level of zoophagy and diet specialisation had significant differences in the level of cannibalism. The zoophagous line exhibited higher level of cannibalism than the phytophahous line, regardless of

whether or not alternative resources were available, a result that supports our first hypothesis. Furthermore, contrary to our second hypothesis, the zoophagous line decreased its cannibalism intensity in response to the presence of both prey and pollen alternative resources. Nonetheless, the response to resource availability was different between the two lines; the phytophagous line only decreased cannibalism when the preferred resource (i.e. the pollen) was available.

Given that conspecifics can serve as a surrogate for prey during periods of prey scarcity and that cannibalism can regulate zoophytophagous predator's population (Leon-Beck et al. 2007), a potential increase in cannibalism in absence of prey would be theoretically beneficial to crop production. However, we observed that protein-rich plant resource (i.e. pollen) strongly prevent cannibalism in zoophytophagous mullein bugs. The consequences of the absence of prey would be important; mullein bug population should remain relatively high and close to the flower (or newly formed fruitlets) where they cause significant damages (Kain and Agnello 2013). Thus, as long as pollen is available to nymphs, cannibalism would only constitute a marginal factor affecting mullein bugs density. However, pollen is usually not available (apple bloom being shorter than bug nymphal development) to the late nymphal stages (e.g. fourth and fifth stage) of the first generation of mullein bug, which growth on apple tree (Bartlett 1996). These stages would have the choice to feed on developing fruitlets (available after petal fall), a low quality resource (O. Aubry, unpublished data), or on conspecifics when preys are not available. Feeding on conspecifics rather than on fruitlets would have considerable consequences on mullein bug populations and consequently on the level of damages caused by nymphs on apple fruit.

The differences in cannibalism between the prey-specialized and the pollen-specialized lines in zoophytophagous mullein bug suggest an additional interest for using more zoophagous lines on crop production. The prey-specialized line potentially provided higher benefits in crop by preferentially choosing prey over plant

resources (Dumont et al. Chap. 2) and by exhibiting high level of zoophagy (Dumont et al. Chap. 3). Additionally, high propensity to cannibalism when the main resources are unavailable (i.e. spider mites and pollen) would decrease mullein bug population density and divert nymphs from feeding on apple fruitlets during a period of risk of damages to crop by mullein bug. For instance, Pels and Sabelis (1999) observed that the predatory mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) delayed dispersal from feeding patch when they had cannibalism opportunities. Mullein bug nymphs that preferentially feed on prey should be found on apple leaves rather than on the flower, and could delay dispersal to flower when they can prey on conspecifics. Thus, applying artificial selection on a zoophytophagous predator population, favouring preference for prey and high level of aggressiveness, could theoretically increase their benefits (increase zoophagy on agricultural pests) while decreasing the risk associated with their presence on crop (by increasing cannibalism during period of high risk of damages to crop). The manipulation of the composition of mullein bug population through artificial selection could be a manner to take advantage of this predator in apple orchards (Hoy 1979; Segal and Glazer 2000).

4.6 Acknowledgement

Grateful thanks to Olivier Aubry for his precious help during rearing settings, and to Pascale Ayotte, Andréanne Lavallée, Laurent Fraser, Sheena-Émilie Boucher and Maïlys Allirand for their help on the capture of individuals, maintenance of isogroup lines and behaviour tests. F.D. was supported by a NSERC PhD fellowship. The project expenses were financed by NSERC discovery grants program allowed to E.L and D.R.

CHAPITRE V

SELECTION OF HIGHLY ZOOPHAGOUS ISOGROUP LINES OF THE MULLEIN BUGS, *CAMPYLOMMA VERBASCI* TO IMPROVE CONTROL OF SPIDER MITES IN APPLE ORCHARDS.

Dumont, F., Réale, D. & Lucas, E.

5.1 Abstract

Zoophytophagous predators provide benefits in agro-ecosystem when feeding on pest species, but they may also damage crops when feeding on vegetal resources. Therefore, the potential of zoophytophagous predators as biological control agents is often neglected. Enhancement of zoophytophagous predators would require improving benefits and/or limiting incurred damages. Recent evidence showed that populations of a zoophytophagous species might actually be composed of a mix of individuals diverging in the level of specialization of their diet, ranging from a mainly phytophagous to a mainly zoophagous diet. Consequently, depending on their level of zoophagy, individuals could vary widely in the benefits and risk they provide to an agro-ecosystem. We tested the hypothesis that manipulating the composition of the population of a zoophytophagous insect, the mullein bug, *Campylomma verbasci* (Hemiptera: Miridae), towards an increased zoophagy would increase the net benefit of their actions in an apple orchard. Using sleeve cages over apple tree branches, we experimentally compared inherent benefits and risks of two different isogroup lines of mullein bug that genetically differed in their level of zoophagy (HZ: highy-zoophagous line; LZ: lowly-zoophagous line). We first compared the level of damages on McIntosh apple fruits by the two lines, during the spring. Second, we

tested for the effect of the two lines, both free-ranging and sleeve-caged, on two-spotted spider mites *Tetranychus urticae* (Acarina: Tetranychidae) populations during the summer. In spring, both strains infrequently punctured apple fruit leading rarely to damage and representing low level of risk on McIntosh. During summer, HZ line lowered spider mite population more efficiently than LZ line or control treatment (no mullein bug) in both sleeve cages and free-ranging tests. Mullein bug's HZ lines, thus, provided extra net benefits that may improve pest control program.

5.2 Introduction

Zoophytophagous predators can surrogate prey by vegetal food items to deal with prey shortage or to complement animal diet (Coll and Guershon 2002). These predators frequently encountered in numerous agricultural systems (Fauvel 1999), can reduce pests populations considerably (Urbaneja et al. 2012). For instance, the release of zoophytophagous predators *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) decreased white fly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) population by about 90 % (Calvo et al. 2009, see also Calvo et al. 2012) and regulated populations of tomato borer *Tuta absoluta* (Meynick) (Lepidopetra: Gelechiidae) (Mollá et al. 2011). Moreover, the capacity to substitute phytophagy for zoophagy allows zoophytophagous predators to survive in agricultural systems during prey shortage and to overcome rapid spatial and temporal changes in their prey availability (Eubanks and Denno 1999; Coll and Guershon 2002). However, prey scarcity and potentially dry meteorological conditions leads zoophytophagous predators to feed on leaves or fruits, which may result in damages to crop (Lucas and Alomar 2002b; Calvo et al. 2009; Arnò et al. 2010), and complicate pest management in agro-ecosystems (Hunter 2009). Therefore, the use of zoophytophagous predators as biological control agents requires the development of approaches maximizing the benefits provided by these predators and minimizing the associated risk (Castañé et al. 2011).

Recently we reported genotypic variation in foraging behaviour of the zoophytophagous mullein bug, a mirid frequently observed in Canadian apple orchards (Dumont et al. Chap. 2, 3). These genotypic differences in foraging included variation in the level of zoophagy (Dumont et al. Chap. 2) and food specialization for either animal or vegetal resources (Dumont et al. Chap. 3). Various nutritional strategies could coexist in mullein bug populations including at one extreme of the range, HZ genotypes (HZ: highly-zoophagous line) relying mainly on prey to meet

their energy requirements, and at the other extreme, LZ genotypes (LZ: lowly-zoophagous line), preferentially feeding on vegetal resources such as pollen. Consequently, some individuals are potentially more beneficial in crop production (i.e. those HZ genotypes) and, thus, would improve the benefit/risk ratio related to the use of zoophytophagous predators.

Genetic improvement of mullein bug could improve the biocontrol of two important apple tree pests, the European red spider mites *Panonychus ulmi* (Koch) (Acari: Tetranychidae) and the two-spotted spider mites *Tetranychus urticae* (Koch) (McMullen and Jong 1970; Thistlewood et al. 1990). Both red and two-spotted spider mites can cause massive damages late in the season during pre-harvest period: reduction of apple fruit size, downgrading and crop losses the following season (Beers et al. 1987). Mullein bug nymphs are the earlier predator of red spider mites; the first generation emerged early in the growing season, and is synchronized with both of apple trees bloom and the first generation of red spider mites (Parent 1973; Thistlewood et al. 1990; Arnoldi et al. 1992). Early season predators usually have a considerable impact on pest population for the whole season of production (Murdoch et al. 1985; Landis et van der Werf 1997; Symondson et al. 2002). Moreover, the beneficial effect of mullein bugs can last for the whole summer as two generations can be observed along the season (McMullen and Jong 1970).

The beneficial role of mullein bug in apple orchards, however, has been neglected because of its potential crop damages (i.e. dark corky wart surrounded by a depression at the surface of the fruit) that it may cause when feeding on apple fruits (Boivin and Stewart 1982). Damages cannot be discernible once the fruit reaches a size larger than ~10 - 13 mm (Kain and Agnello 2013; Aubry et al. 2015). Therefore, only nymphs of the first generation cause damages to apples. The period of potential damages by the mullein bug, thus, last from full bloom until the fruit reach about 13 mm (Boivin and Stewart 1982; Reding et al. 2001; Kain and Agnello 2013).

Moreover, most apple cultivars are tolerant to mullein bug's punctures as only Red and Yellow Delicious are very threatened (Aubry et al. 2015). Since damages on apple fruits happen over a short period (about two to three weeks) and that benefits can extend over the entire apple production period, it could be worth considering methods that allow exploiting the mullein bug potential as a biocontrol agent.

In this study, we first tested the hypothesis that highly-zoophagous mullein bug line (HZ line) generates less damages to apple than lowly-zoophagous line (LZ line). In spring, we investigated whether there were differences in the number of punctures observed on apple fruitlets between a HZ and a LZ lines in absence of prey. In this circumstance, mullein bugs are potential pest on apple tree (Kain and Agnello 2013), but diet specialisation (Dumont et al. Chap. 3) and variation in cannibalism (Dumont et al. Chap. 4) could induce differences in number of punctures. Secondly, the hypothesis that HZ line provides more benefits in apple orchards by having a greater impact on spider mites than LZ line has been tested. During summer, we compared the effect of mullein bugs on spider mites population both in sleeve cages and free-ranging set-ups.

5.3 Materials and Methods

5.3.1 Populations and rearing

In experiments 1 and 2, we used mullein bug nymphs from two isogroup lines selected from a group of twelve lines raised in our laboratory. Individuals at the origin of the isogroup lines were captured on apple trees and mullein plants, either as eggs (in December 2011) or as adults (in summer 2011 and 2012). Each isogroup line was initially composed of two virgin males and two males and maintained during nine generations (assuming a generation every 40 days). It was held in acrylic glass cage (30 X 30 X 30 cm) containing one mullein plant, one soybean plant and two

potato plants. Green peach aphids, two-spotted spider mites and pollen were provided *ad libitum*. More details on the set-up and maintenance of the lines are provided in Dumont et al. (Chap. 2, 3). In this series of experiments, we picked up both the lines with the highest and the lowest level of zoophagy (hereafter referred to as HZ = high-zoophagy and LZ = low-zoophagy line) on two-spotted spider mites (see Dumont et al. Chap. 2).

To produce a large number of individuals necessary for the experiments HZ and LZ lines were reared in five muslin cages containing six potatoes plant infested with aphids. Mullein bugs were also provided with *ad libitum* pollen and a solution of sugar and distilled water. The cages were kept in a greenhouse at ~30°C, ~ 60% R.H. and 16 hours of daylight. The first experiment on damages was run one generation (~40 days) after the establishment of massive rearing, whereas the second experiment on benefits was conducted at the third generation.

5.3.2 Apple orchards and experiments

Both experiments were run during the summer 2013. Experiment 1 was run at an apple orchard located in Saint-Joseph-du-Lac (Laurentians, Quebec, Canada) (45° 32' 00" N; 74° 00' 00" W). Experiment 2 took place at an orchard localised in Rougemont (Monteregie, Quebec, Canada) (45° 26' 00" N; 73° 03' 00" W). We switched the orchard for experiment 2 because the first orchard was not infested by spider mites in August (a condition required to run experiment 2). During both experiments, neither insecticides, nor acaricides were used on the treated trees. Fungicides were applied as normal in the orchard.

In the first experiment we compared damages produced by HZ and LZ lines on apples from a single McIntosh apple tree during the bloom (2013-05-18). Forty muslin sleeve cages (20 X 20 X 70 cm) were installed on as many branches with at least two

flower clusters. Branches were previously inspected and cleaned from all arthropods. Flowers were manually pollinated with a fine paintbrush to ensure fruit development. A pair of young nymphs (L1 and/or L2) of either the HZ or the LZ line was introduced in each sleeve (i.e. 20 HZ and 20 LZ). Sleeves remained closed until the end of June (2013-06-27). On that day, apple fruitlets in each sleeve were collected for laboratory analysis. We inspected 54 and 52 fruitlets (including drops), for the LZ and the HZ lines, respectively. In laboratory, fruitlets were thoroughly inspected with 40 X binocular magnifiers and the number of punctures (damages) on each of them was counted. At this stage of the apple fruit development, mullein bug punctures are characterised by a small circular depression on the surface of fruitlet (see Boivin and Stewart 1982). Apple fruitlets were rated following Kain and Agnello (2013): fruit with a single puncture or less (0 or 1) (U.S. Fancy grade); two punctures or more ($\geq$ 2) (downgraded). We also noted whether the fruitlet was dropped or not. All apple fruitlets, dropped and non-dropped, were weighted.

An additional seven sleeve cages were installed as control treatment (without mullein bug nymphs). On the 19 apple fruitlets found in control treatment's sleeves, only one had a single damage similar to a puncture by a mullein bug (some mullein bug may have hatched after the installation of our sleeves).

In the second experiment we compared the efficiency of HZ vs LZ lines to control spider mite population growth. For this, we used both sleeve-caged (see above) and free-ranging nymphs in apple orchards. In 14 sets of three apple trees (Spartan cultivar), each separated by a buffer tree, we installed sleeve cages containing HZ, LZ lines, or no bugs (i.e. control tree). The control tree was always placed in the middle of the set. On each tree, four branches were selected (one on the four sides of the tree): one was equipped with a sleeve cage and the three others for the free-ranging test. Prior to setting up the sleeve cage, we removed any insect present on the branch, with the exception of spider mites. The populations of two-spotted and red spider

mites were estimated on each branch by counting every mobile form of both species on the bottom side of five randomly selected leave (three leave for the sleeve-cage test). For the free-ranging test, the populations of spider mites were estimated on the day of introduction (day 0; 14 August 2013), on day 7 (21 August 2013) and on day 21 (4 September 2013). The estimation of spider mite's populations for the sleeve-cage test was done on the day of introduction (day 0; 18 August 2013) and on day 14 (1st September 2013). Red spider mites were very scarce and were not considered in our analysis. We observed a very low number of predatory mites (i.e. Phytoseiids). While we did not run standardized sampling of mullein bugs in the orchard, we observed the presence of both nymphs and adults from the natural population.

The three treatments consisted of 1) a control without added mullein bug nymphs, 2) a HZ line treatment and 3) a LZ line treatment. In the sleeve cage tests, three nymphs (N3 to N5 stages) were introduced per sleeve cage, whereas four nymphs (N3 to N5) were released on each experimental branch in free-ranging tests.

5.3.3. Statistical analysis

Experiment 1. The probabilities of an apple fruitlet to be downgraded ($\geq$ 2 mullein bug' punctures) in function of the treatment (HZ or LZ line) were tested implementing a generalized linear model (GLM) for binomial distribution (0 = 'fancy', 1 = downgraded). The proportion of cluster with damages was compared between treatments using a GLM model for binomial distribution. A GLM for binomial distribution was used to compare probabilities of an apple fruitlet to be dropped among treatments (control, HZ, or LZ line). The number of punctures in the dropped fruitlets as a function of treatment (HZ or LZ line) was tested using a GLM model for count data (Poisson distribution accounting for overdispersion). The mean weight of non-dropped apple fruitlets found in each sleeve cage was compared among treatment using GLM model for Gaussian distribution.

Experiment 2. In sleeve cage tests, the two-spider mites populations were compared among treatments (control without mullein bug, HZ, or LZ line) using analyse of covariance (ANCOVA) and post-hoc (using the *glht* function; package *multcomp*). The initial number of spider mites was added as a covariate. For the free-ranging test, a linear mixed model was run to test the number of spider mites after 7 and 21 days according to the treatment and the initial population. Since three measures were taken on each tree, the variable tree ID was included as a random effect in the model. We estimated the highest posterior density (HPD) intervals for the LMM model from a posterior distribution of fixed effect parameters generated with the *mcmcsamp* function (*lme4* package).

All statistical analysis has been run on R (R Core Team 2013).

5.4 Results

5.4.1 Experiment 1. Damages on apple fruitlets.

In the HZ line treatment 94.4% (17 out 18 fruitlets) of the apple fruitlets were classified as 'fancy' (one or no puncture), which did not differ from the 90.6 % (29 out 32 fruitlets) in LZ line treatment ($\beta = -0.56 \pm 1.19$; $z = -0.47$; $p = 0.64$).

The proportion of clusters showing damages were not different between HZ lines (36.8 ± 49.6 %) and the LZ lines (55. 6 ± 51.1 %) ($\beta = -0.76 \pm 0.67$; $z = -1.14$; $p = 0.26$).

The rate of fruitlet drops was 26.3 % in the control, 40.7 % with LZ lines, and 65.4 % with the HZ treatment. There were significantly more drops in sleeve cages with the HZ line than in the control treatment ($\beta = 1.67 \pm 0.60$; $z = 2.79$; $p = 0.005$) (Figure

5.1). The probability of drops with the LZ line was not significantly different from the control treatment ($\beta = 0.65 \pm 0.59$; $z = 1.11$; $p = 0.27$).

The mean number of punctures observed on dropped fruitlets was 0.32 (± 0.98) for the HZ line and 0.45 (± 1.06) for the LZ line, and did not differ between the two lines ($\beta = -0.28 \pm 0.49$; $z = -0.56$; $p = 0.58$).

Non-dropped apple fruitlets weighted 9.84 (± 4.05) g in the control treatment whereas it was 12.51 (± 7.15) g in treatment with HZ lines and 10.25 (± 8.00) g for the LZ line. Neither the LZ line ($\beta = 0.07 \pm 0.46$; $z = 0.15$; $p = 0.88$) nor the HZ line ($\beta = 0.50 \pm 0.48$; $z = 1.05$; $p = 0.30$) differed from the control treatment.

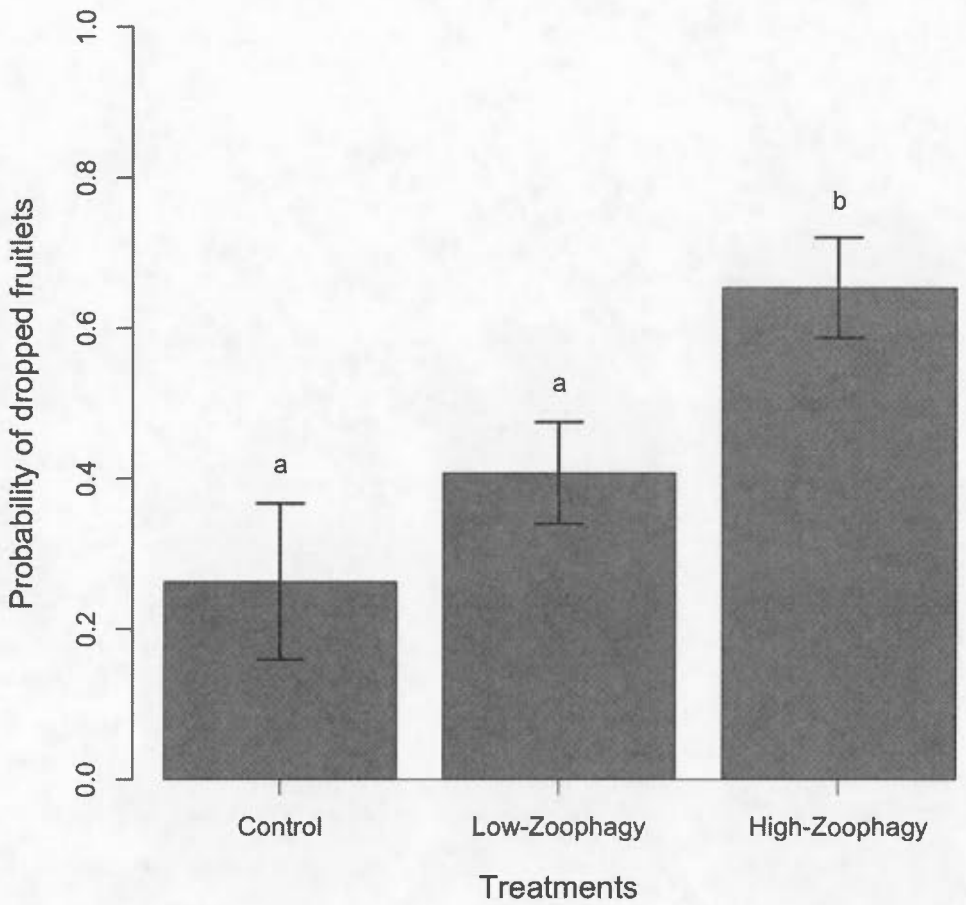

Figure 5.1. Probability of dropped fruitlets in function of three treatments: control (without mullein bug), two lowly-zoophagous mullein bug nymphs and two highly-zoophagous mullein bug. Means with the same letter are not statistically different.

5.4.2. Experiment 2. Benefits on the control of spider mites

In sleeve cages, the initial mean number of two-spotted spider mites per leaf did not differ among the control treatment (19.86 ± 8.19 s.d.), the HZ treatment (18.88 ±

12.18) and the LZ treatment (26.40 ± 10.86) (HZ line: $\beta = -0.12 \pm 0.24$; $z = -0.52$; $p = 0.61$; LZ line: $\beta = 0.38 \pm 0.24$; $z = 1.61$; $p = 0.12$). At day 14, the number of mites differed significantly among the average 89.9 (± 58.85) mites.leaf^{-1} counted in the control treatment, 48.29 (± 34.05) mites.leaf^{-1} in the HZ treatment, and 71.05 (± 44.41) mites.leaf^{-1} in the LZ treatment ($F_{2,38} = 5.71$; $p = 0.007$) (Figure 5.2). Mites were less abundant in the HZ treatment that in the control one, whereas the LZ treatment did not had statistically significant effect. The initial number of mites did not have an effect on the number of mites at the end of the test ($F_{1,38} = 1.75$; $p = 0.19$).

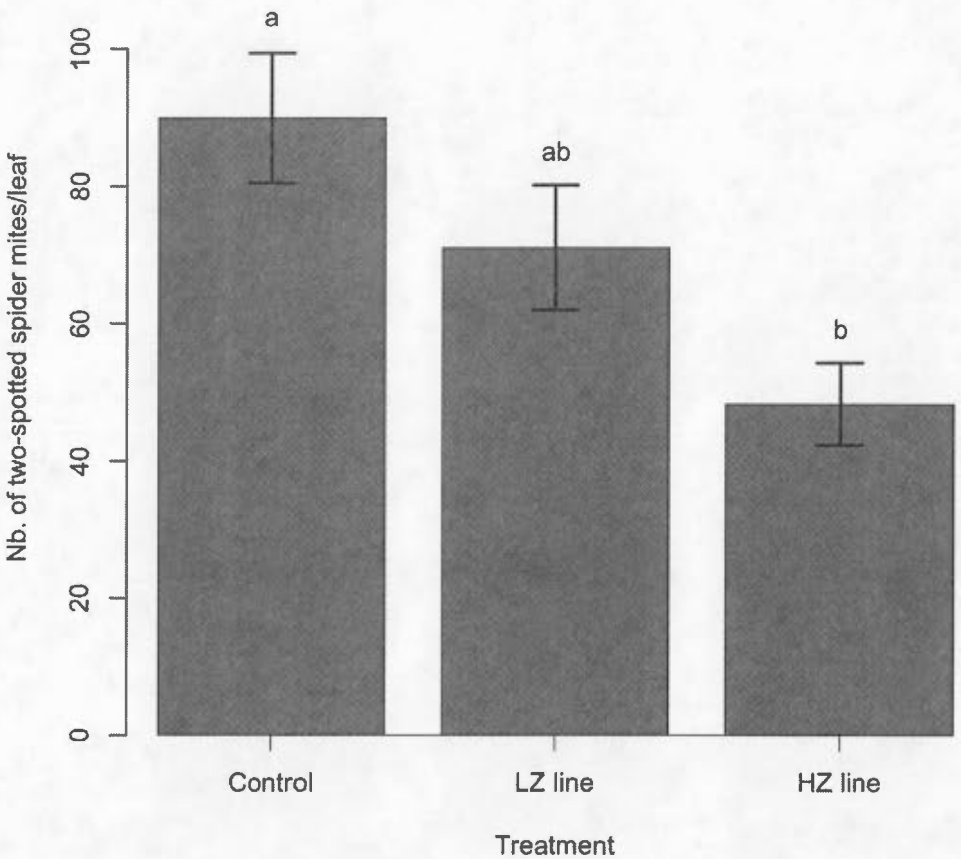

Figure 5.2. Number of two-spotted spider mites 14 days after the introduction of mullein bug nymphs in sleeve cages. Means with the same letter are not statistically different. Error bars are standard error.

In the free-ranging test, the initial two-spotted spider mites population was 20.56 (± 12.68 s.d.) individuals by leaf in the control treatment, 22.94 (± 20.0) in the LZ treatment and 22.61 (± 14.47) in HZ treatment. There was no difference among

treatment in their initial population (LZ line: β = 0.12; 95 % HPD = [-0.59; 0.88]; HZ line: β = 0.20; 95 % HPD = [-0.57; 0.90]).

At day 7 we counted on average 28.39 (± 15.89) mites.leaf^{-1} in the control, 26.77 (± 16.38) mites.leaf^{-1} in the HZ, and 27.19 (± 15.41) mites.leaf^{-1} in the LZ treatment (Figure 5.3), which was not statistically different (LZ line: β = -0.18; 95 % HPD = [-0.80; 0.50]; HZ line: β = -0.27; 95 % HPD = [-0.92; 0.37]). The number of mites after 7 days depends on the initial mite's population (β = 0.006; 95 % HPD = [0.002; 0.009]).

At day 21 the number of two-spotted spider mites per leaf had increased to 100.82 (± 42.31) in the control, to 81.38 (± 25.71) in the HZ, and to 89.15 (± 32.72) ind./leaf in the LZ treatment (Figure 5.3). The HZ line decreased significantly the number of spider mites compared to the control treatment (β = -0.93; 95 % HPD = [-1.74; -0.11]), but it was not the case to the LZ line (β = -0.54; 95 % HPD = [-1.43; 0.24]). The HZ line lowered the growth of the spider mite's population by 26.8 %, while LZ line only lowered the growth of their prey population by 17.5 %. The initial number of mites did not had an effect on the number of mites after 21 days (β = -0.0001; 95 % HPD = [-0.002; 0.006]).

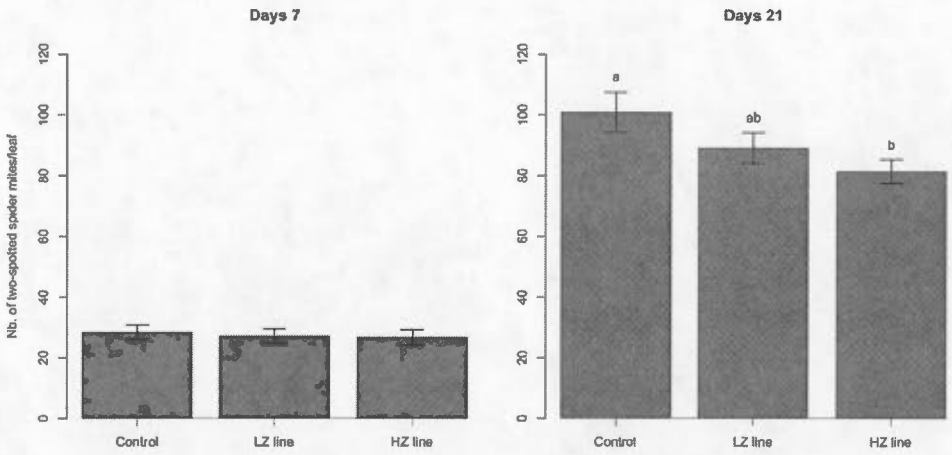

Figure 5.3. The number of two-spider mites 7 and 21 days after the introduction of free-ranging mullein bug nymphs

5.5 Discussion

In agro-ecosystem, the benefits provided by zoophytophagous predators feeding on pests can be thwarted by the damages they caused by foraging on crop (Cohen 1996; Castañé et al. 2011). Biological control management programs promoting the use of zoophytophagous predators must thrive to increase the ratio of benefits/risks associated with such agent (Castañé et al. 2011). Genetic differences in foraging behaviour/strategy provide an opportunity to increase benefits and to decrease risks either by artificially selecting traits of interest, or by agricultural practices favouring certain genotypes in a population of zoophytophagous insects (Dumont et al. Chap. 2, 3). Our results indicate that the more zoophagous mullein bug line (HZ) had a stronger impact on two-spotted spider mite's populations than the least zoophagous line (LZ). Thus, genetic differences in foraging behaviour of mullein bugs observed in the laboratory (Dumont et al. Chap. 2, 3) reflected difference in the benefits they provide to apple production. In addition, both lines generated low level of punctures

to apple fruitlets. The HZ line also increased the rate of apple fruitlet drops compared to the LZ line. Although we do not have precise explanation for this effect, that result indicates that high zoophagy in mullein bugs might be used with profit by selecting lines which can act as a natural thinning agent (Sanchez and Lacasa 2008). However, dropped fruitlets were not markedly punctuated by mullein bug contrary to what reported by Kain and Agnello (2013). Kain and Agnello (2013) observed more dropped fruitlets when mullein bugs were not removed from apple tree until after petal fall. Perhaps the higher quantity of dropped fruitlets in HZ line's sleeve cages arises from differences in plant parts consumed (e.g. pollen, ovaries, fruitlets, leaves) or in the timing of feeding (early or later). Further studies would be required to clearly see the relationship between dropped fruitlets and mullein bug's feeding habits. Taken together, the extra benefits obtained from HZ line during summer and the low risk in spring increase the ratio of benefits/risks associated with zoophytophagous mullein bug in apple orchard. Artificial selection programs could thus profit of that genetic variation to improve zoophytophagous predator impact (Hoy 1979; Hopper et al. 1993; Segal and Grazer 2000).

Releasing highly zoophagous mullein bugs during pre-harvesting period would have both short-term and long-term benefits in apple orchards. On short-term basis, mullein bugs attack spider mites, at a time when producers stop to spray pesticides, and thus limit the risk of spider mite outbreaks. Note that, in our experiment, only 3 to 4 mullein bugs nymphs per branches had a significant effect on the number of mites, with a 20 % decrease in presence of HZ mullein bugs compared to the control treatment in the free-ranging (and 46 % in the sleeve cages). This indicates that releasing a relatively small quantity of highly zoophagous mullein bugs could be enough to keep spider mite's population under a level that could control its damage. On the long-term, releasing HZ line will increase the frequency of these genotypes within the natural mullein bug's population. In Dumont et al. (Chap. 3), we suggested that the variation in the status of zoophytophagous predators from a region to another

was possibly due to different genetic composition of their populations. Beneficial populations could be composed of HZ genotypes, whereas noxious population mainly of LZ genotypes. The release of artificially selected mullein bugs could thus turn a pest population into a beneficial one.

Risks associated with zoophytophagous predators increase when their density is high relative to the density of their prey (Sanchez 2008; Arnó et al. 2010). However, the level of damages to fruitlets was very low in our experiment even if mullein bug nymphs did not have access to prey. Our results contrast with those of Kain and Agnello (2013), even if our experiment was modelled on that study (i.e. two young nymphs caged on McIntosh's flower clusters at bloom). Kain and Agnello (2013) observed damages in 87 % of cluster when young mullein bug nymphs were introduced at bloom, whereas we observed that the percentage of clusters damaged was ~56 % in the LZ treatment and ~37 % in the HZ treatment. This difference among results is consistent with the hypothesis that the difference in the status of mullein bug from a region to another is related to a variation in the composition of the population (Dumont et al. Chap. 3). In Quebec, some authors consider mullein bugs as predators that can provide some benefits in apple orchards (Boivin and Stewart 1982; Arnoldi et al. 1992), whereas in New York State they are identified clearly as a pest (A. Agnello, personal communication). These variations between regions suggest that agricultural practices (e.g. use of broad spectrum pesticides) or ecological factors (e.g. availability of alternative herbaceous hosts near orchards) may have created the variation in the composition of mullein bug population in term of individual using a zoophyphagous or a phytozoophagous strategy (Dumont et al. Chap. 3). The effect of both ecological and anthropic factors affecting the composition of mullein bug's population needs to be investigated in order to obtain long-term success with a biological control program of spider mites using artificially selected mullein bugs.

Our results support the idea that genetic variation within zoophytophagous predator's population should be considered in biological control program based on these predators. The role of zoophytophagous predators has been studied extensively in the tomato crops. In Europe, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and *N. tenuis* are commercially sold as biological control agents of whiteflies and tomato borer (Castañé et al. 2011). Commercialising genetically improved lines could improve this approach. For instance, Nachappa et al. (2011) observed that genetic variation in prey consumption, conversion efficiency and dispersal in predatory mite

Phytoseiulus persimilis (Athias-Henriot) (Acarina: Phytoseiidae) affect predator-prey interactions, long-term population dynamics as well as efficiency in the biological control of two-spotted spider mites. Genetic improvement of predator used as biological control agents earn scant attention despite the need to consider the genetic aspect is recognize for long time (Hoy 1979; Roush 1990). Most studies focused on pesticide susceptibility of biological agents rather than trait related to their efficiency in field. However, our study and that of Nachappa et al. (2011) demonstrated the potential of genetic improvement of predators in attributes that affect success in biological control. In the specific case of zoophytophagous predators, genetic improvement could target simultaneously zoophagy and phytophagy to increase the benefits and reduce the risk associated to these predators.

In the present study, we used the isogroup line's method rather than running artificial selection to obtain two populations of mullein bugs that vary in traits of interest (i.e. zoophagy and food specialization). The isogroup line's has the advantage to obtain improved lines rapidly (since the first generation). Improvement could remain for several generations and hold despite extensive mass rearing and diet (Castañé et al. 2002). In our case, we kept isogroup lines for about fifteen generations to support experiments presented in Dumont et al. (Chap. 2, 3 and 4). The HZ lines were consistently more zoophagous than the LZ lines during all this period. In addition,

isogroup or isofemale lines are simple methods to invest the genetic variation within a population, and determine the frequency of HZ genotypes (David et al. 2005). Most individuals used to implement our isogroup lines were captured as eggs during winter. It could thus be possible to capture individuals during winter, set up isogroup lines and determine the frequency of HZ genotypes before the natural population emerge during apple tree flowering. This information would be useful to plan management of mullein bugs in apple orchards. Alternatively, artificial selection would require more time to implement (several generations). For instance, Nachappa et al. (2010) selecting for high and low voracity of the predatory mites *Phytoseiulus persimilis* reach stability in the trait after two or three generations. However, this approach could improve lines more significantly than do isogroup lines. At each generation, selected individuals would bring the lines a bit further in the desirable direction. Both isogroup lines and artificial selection should be carefully applied as deleterious gene could be unwittingly selected during the process (Hill and Caballero 1992). Inbreeding depression, deleterious effects of inbreeding such as reduced fecundity, longevity, changes in sex ratio and male sterility, remained a potential threat in laboratory-reared or artificially selected populations (Hopper et al 1993).

5.6 Acknowledgements

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CHAPITRE VI

CONCLUSION

La problématique des prédateurs zoophytophages en milieu agricole a suscité beaucoup d'intérêt dans les 30 dernières années (Coll et Guershon 2002; Castañé et al. 2011). Plusieurs aspects de ces espèces ont été étudiés, notamment les préférences alimentaires, le comportement de changement trophique, la réponse fonctionnelle à la disponibilité des ressources et les effets sur les traits d'histoire de vie (Alomar et Wiedenmann 1996; Coll et Ruberson 1998; Coll et Guershon 2002; Symondson et al. 2002). Néanmoins, cette problématique n'a pas été approchée sous l'angle des différences individuelles ou génétiques. Mon étude sur les punaises de la molène montre qu'il serait possible de tirer profit des différences génétiques dans les caractères d'intérêts économiques comme la zoophagie et la spécialisation alimentaire. En outre, j'ai observé que les lignées isogroupes varient considérablement dans leur niveau de zoophagie, et que cette variation pouvait être associée à des stratégies zoophytophages et phytozoophages. L'utilisation d'une lignée plus zoophage a permis d'augmenter les bénéfices de ce prédateur dans la lutte aux tétranyques en fin de saison. De plus, mes résultats apportent des nouvelles connaissances sur l'écologie des punaises de la molène, montrent que les informations sur la génétique de cette espèce peuvent éclairer certains aspects de sa biologie parfois difficile à comprendre (ex : les différences de statut selon les régions et leur causes potentielles), et génèrent des nouvelles pistes de recherche sur l'amélioration génétique des agents de lutte biologique. Bien entendu, une telle étude n'a pas pu aborder toutes les facettes d'un programme sur la punaise de la molène. Cette conclusion porte donc sur les différentes pistes de recherche qui pourraient être approfondies dans le futur.

6.1 Punaise de la molène

Les ressources disponibles aux punaises de la molène varient considérablement durant la vie d'un individu, mais aussi d'une génération à l'autre. Mes résultats montrent que la punaise de la molène peut répondre aux contraintes en matière de ressources alimentaires en modifiant sa diète. Cependant, ils montrent également l'existence de différences génétiques dans l'exploitation et le choix des ressources. Certaines lignées tuaient significativement plus de tétranyques à deux points que d'autres. En présence de tétranyques et de pollen, certaines lignées consommaient préférentiellement les proies alors que d'autres consommaient plutôt le pollen. De telles variations dans l'exploitation et la sélection des ressources suggèrent l'existence d'un certain degré de spécialisation alimentaire dans les populations de punaise de la molène. Ainsi, les individus devraient adapter leurs stratégies d'approvisionnement et d'oviposition en fonction de leur spécialisation alimentaire. Les choix de site de vie et de ponte des individus devraient donc être basés sur la disponibilité des tétranyques ou du pollen sur chacun de ses différents hôtes végétaux.

La spécialisation alimentaire devrait influencer le choix de site d'oviposition par les femelles adultes et l'utilisation de l'habitat par les larves de punaises. La première génération (printanière) de punaise de la molène émerge à la floraison des pommiers. À ce moment, les larves disposent de pollen de pommier et, parfois, de tétranyques rouges. Malgré leur proximité, le pollen se trouve dans les fleurs, à un emplacement différent des tétranyques principalement localisés sur les feuilles. Thistlewood et McMullen (1989) observent que les jeunes larves de punaises de la molène sont fréquemment observées près des fleurs de pommier au printemps, alors qu'elles sont très rares sur les feuilles. Ils suggèrent que, du fait de leur petite taille et de leur délicatesse, les larves de premier stade sont peu enclines à se déplacer des fleurs aux feuilles (et vice versa). Ainsi, la distribution des larves au printemps dépendrait du comportement d'oviposition de leur mère à l'automne. La sélection non-aléatoire des

sites d'oviposition est fréquente chez les mirides arboricoles (Sandford 1964; Lord 1965). Les femelles seraient en mesure de discerner les bourgeons floraux des bourgeons végétatifs (Thistlewood et McMullen 1989). Cependant, la présence de tétranyques sur les pommiers à l'automne influencerait aussi le choix d'oviposition des femelles (Thistlewood et McMullen 1989). Les femelles seraient donc influencées par différents stimuli et pourraient y répondre différemment selon qu'elles sont spécialisées sur les ressources animales ou végétales. Pour la deuxième génération, dite estivale, la réponse des femelles aux stimuli (ex. disponibilité d'hôtes herbacés ou de proies) aurait des conséquences importantes sur le type de ressources disponibles pour leurs larves. La génération estivale émerge en juillet sur les pommiers (ou autres arbres rosacés) ou sur des hôtes herbacées comme la molène *Verbascum* spp. (mais aussi la pomme de terre *Solanum tuberosum*, la morelle noire (*Solanum nigrum*), le maïs sucré *Zea mays* et le sumac vinaigrier (*Rhus typhina*) (McMullen et Jong 1970; Arnoldi et al. 1992). Les tétranyques et le pollen peuvent ne pas être disponibles simultanément sur ces hôtes en juillet. Par exemple, la molène a une période étendue de floraison, mais les tétranyques exploitent rarement ses feuilles très pubescentes, tandis que les tétranyques sont souvent abondants sur les pommiers en été au moment où le pollen n'est plus disponible. Ainsi, les ressources disponibles pour les larves de la génération estivale dépendront du comportement d'oviposition (choix de l'hôte) des femelles de la génération printanière. Notamment, les femelles zoophytophages (sur pommiers) devraient pondre sur des sites où les larves vont trouver des proies abondantes, tandis que les femelles phytozoophages (sur molène) chercheront des sites où le pollen sera abondant. Il faut néanmoins noter que les tétranyques à deux points sont très polyphages et exploitent plusieurs hôtes dont certains (ex. les fraises) pourraient offrir à la fois tétranyques et pollen aux punaises.

Lors de mon projet, j'ai récupéré des individus de différents sites, récoltés sur différents plants de pommiers et de molène et à différentes périodes. Pour le moment nous ne savons pas si 1) les génotypes zoophytophages ou phytozoophages sont

répartis de manière différente selon les plants hôtes et les sites de récoltes. Il serait donc intéressant de vérifier, dans un futur projet de recherche, s'il y a effectivement un lien entre le choix de l'hôte d'oviposition et la spécialisation de diète.

Le risque de prédation et la compétition interspécifique associés aux différentes ressources et hôtes disponibles pour les punaises de la molène peuvent être très variables. Les larves de punaise de la génération printanière auront de la compétition par des acariens prédateurs ou par la coccinelles acariphages *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) pour leur principale ressource animale soit les tétranyques rouges (Parent 1973), alors qu'elles retrouveront le thrips des petits fruits *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) et la coccinelle maculée *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) sur les fleurs de pommier. Ainsi, des variations dans les populations de ces compétiteurs pourraient favoriser une spécialisation plutôt qu'une autre. De plus, la punaise de la molène peut être impliquée dans des interactions de type prédation intraguilde avec plusieurs de ces compétiteurs. La prédation intraguilde est un type d'interaction écologique dans laquelle une espèce, le prédateur intraguilde, se nourrit d'une espèce, la proie intraguilde, avec laquelle elle partage une ressource alimentaire commune (Polis et al. 1989). La punaise de la molène peut jouer le rôle de prédateur intraguilde en attaquant, par exemple, les différents prédateurs acariens, mais elle peut à son tour être victime de prédation intraguilde notamment par les coccinelles. L'utilisation de l'habitat par les larves, qui pourrait varier selon qu'elles sont spécialisées pour les ressources animales ou végétales, modulerait donc les probabilités de rencontre des différents prédateurs et proies intraguildes. Ainsi, le niveau de compétition inter- et intraspécifique et le risque de prédation associé à chacune des spécialisations pourraient être très différents. Des variations dans ces paramètres écologiques favoriseraient le maintien de génotypes spécialisés sur les ressources animales ou sur les ressources végétales.

Les interactions intraguildes que peuvent entretenir les punaises sont susceptibles d'influencer l'efficacité de la lutte biologique. Les populations de tétranyques en verger peuvent être maintenues sous le seuil de nuisibilité par les prédateurs (Lord 1956). Les prédateurs acariens, notamment *Neoseiulus fallacis* (Garman) (Acarina: Phytoseiidae) et *Agistemus fleshneri* (Summers) (Acarina: Stigmaeidae), sont très utiles dans la lutte aux tétranyques (Chouinard et al. 2001). Cependant, une augmentation du niveau de zoophagie des punaises de la molène pourrait avoir un impact négatif sur les populations de prédateurs acariens, d'une part, en augmentant le niveau de compétition interspécifique et, d'autre part, en augmentant l'incidence de prédation intraguilde par la punaise sur les acariens. Provost et al. (2006) ont observé que le prédateur acarien *A. fallacis* était une proie exploitée par la punaise translucide *Hyaliodes vitripennis* (Say) (Hemiptera: Miridae), bien que cette dernière préfère les tétranyques à deux points (les proies intraguildes dans ce système). Il serait donc important de vérifier si l'utilisation de punaises très zoophages n'engendrerait pas d'effets négatifs sur les prédateurs acariens, ce qui pourrait compromettre la lutte aux tétranyques par les différents ennemis naturels.

6.2 Lutte biologique et amélioration génétique

L'approche par différences individuelles ou génétiques nous permet d'en apprendre davantage sur les impacts écologiques et économiques des prédateurs zoophytophages en milieu agricole. Mes résultats suggèrent que certains individus dans une population de prédateurs zoophytophages pourraient potentiellement procurer plus de bénéfices, alors que d'autres représenteraient un risque plus élevé pour les récoltes. Les différences individuelles ou génétiques pourraient expliquer, au moins en partie, le rôle mitigé des prédateurs zoophytophages. L'ambigüité dans le statut de ces prédateurs d'une région à l'autre pourrait correspondre à des différences de composition des populations. Par exemple, la punaise de la molène est clairement

identifiée comme un insecte ravageur (essentiellement phytophage) dans les vergers de pommiers ou de poiriers en Colombie Britannique et dans l'État de New York (McMullen et Jong 1970; MacPhee 1976; Kain et Agnello 2013). En revanche, les bénéfices que peuvent procurer les punaises de la molène en vergers sont reconnus dans d'autres régions, notamment au Québec (Boivin et Stewart 1982). Conséquemment, certains auteurs qualifient les punaises de la molène comme prédateur zoophytophage (Kinkorova and Kocourek 2000; Reding et al. 2001; Fréchette et al. 2008; Aubry et al. 2011), alors que d'autres la considèrent comme un insecte phytozoophage (Sanford 1964; Torres et al. 1999). L'hypothèse énonçant que les populations de punaises de la molène diffèrent en composition (i.e. certaines populations seraient principalement composées d'individus spécialisés sur les ressources animales, tandis que d'autres compteraient en majorité des individus spécialisés sur les ressources végétales) est cohérente avec mes résultats sur les bases génétiques de la spécialisation alimentaire. Pour que de telles différences entre les populations existent, il est nécessaire que la spécialisation alimentaire soit, au moins partiellement, génétiquement déterminée, et que certains facteurs écologiques et/ou anthropiques favorisent la divergence des populations.

Mes résultats apportent de l'information sur les bases génétiques de la spécialisation alimentaire chez les punaises de la molène, mais les facteurs qui peuvent entrainer un changement de fréquence des différentes spécialisations restent à déterminer. Comme il a été mentionné précédemment, les risques de prédation, la compétition inter- et intraspécifique et surtout la disponibilité des ressources et hôtes végétaux alternatifs sont d'autant de facteurs qui peuvent influencer le succès des spécialisations sur les ressources animales ou végétales. De plus, les prédateurs zoophytophages peuvent être menacés par les traitements chimiques soit par des effets directs sur leur survie et leur reproduction (Moser et Obrycki 2009; Torres et al. 2010; Arnó et Gabarra 2011) ou soit par une réduction de l'abondance de leurs proies (Kinkorova et Kocourek 2000). Chez les punaises de la molène, les femelles de la première génération pondent

leurs oeufs soit dans un hôte herbacé (e.g. les plants de molène) ou dans les pommiers (McMullen et Jong 1970). Les larves émergent sur les pommiers en juillet (stratégie zoophytophage) peuvent subir les effets négatifs de l'application d'insecticides qui visent notamment le carpocapse de la pomme *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), tandis que les larves qui émergent sur les hôtes herbacés (stratégie phytozoophage) ne le seraient pas. Si les femelles spécialisées sur les ressources animales choisissent les pommiers pour y pondre leur oeufs (où les tétranyques sont abondants l'été, mais pas le pollen), les traitements chimiques auront pour effet d'éliminer les individus plus bénéfiques de la population. Ce processus de sélection pourrait rapidement entrainer des changements indésirables dans une population de punaise de la molène considérant l'héritabilité relativement élevée de la réponse des punaises à la disponibilité du pollen ($r = 0.45$). Ultimement, de tels changements dans la composition des populations pourraient mener à des variations aux niveaux des bénéfices et des risques que représente la punaise de la molène en milieu agricole. L'utilisation d'insecticides moins néfastes pour les punaises de la molène et le recours à une lutte biologique plutôt qu'à une lutte chimique contre les tétranyques permettraient de réduire la pression sur les punaises zoophytophages très voraces et d'éviter les dérives possibles. Des changements non désirés dans la composition de la population de punaise de la molène pourraient ainsi être évités.

D'un autre côté, la connaissance des bases génétiques de ces traits d'intérêts économiques (i.e. la zoophagie, spécialisation alimentaire) et des facteurs écologiques offrent l'opportunité de manipuler volontairement la composition des populations de prédateurs zoophytophages afin de valoriser leur utilisation en lutte biologique. Cette idée pourrait être appliquée à l'amélioration génétique des punaises zoophytophages employées comme agent de lutte biologique. Par exemple, il serait possible d'augmenter les bénéfices et de réduire les risques associés aux punaises *Macrolophus pygmaeus* et *Nesidiocoris tenuis* produites commercialement et vendues en Europe pour lutter contre les aleurodes et les larves de mineuses de la tomate *Tuta*

absoluta Meyrick (Lepidoptera: Gelechiidae). Hoy (1979) soutenait que le potentiel de l'amélioration génétique des agents de lutte biologique était presque inexploité dans les années 1970, et cette approche n'a été que très peu développée depuis. Le développement effervescent des connaissances sur les différences individuelles (Réale et al. 2007; Bolnick et al. 2011; Sih et al. 2012; Dall et al. 2012) depuis les années 2000 pourrait offrir un second souffle à l'approche par amélioration génétique des agents de lutte biologique. Le potentiel est considérable puisque les variations entre les individus modifient leurs interactions écologiques et modulent, entre autres, les interactions prédateur-proie et la composition des communautés (Dall et al. 2012). Nachappa et al. (2011) ont démontré que des lignées de l'acarien prédateur *Phytoseiulus persimilis* artificiellement sélectionnées pour augmenter leur voracité, le taux de conversion et leur dispersion variaient dans le ratio de proies/prédateur et dans la corrélation spatiale entre les prédateurs et les proies en serre. Toutes les lignées sélectionnées étaient plus efficaces dans la lutte aux tétranyques à deux points qu'une lignée témoin sans sélection artificielle (Nachappa et al. 2011). Ces résultats suggèrent que la sélection artificielle sur certains traits permettrait d'atteindre des objectifs précis en champs. Par exemple, la lignée très vorace de Nachappa et al. (2011) avait un impact local très fort sur les proies, mais n'avait pas une bonne corrélation spatiale. Cette lignée pourrait donc être utilisée en lutte biologique inondative, pour lutter contre les ravageurs pendant une période donnée. En revanche, les lignées à haut taux de conversion et à dispersion rapide permettraient une lutte à plus long terme contre les ravageurs. Ainsi, la sélection artificielle pour l'amélioration génétique des agents de lutte biologique peut s'appliquer autant aux prédateurs généralistes (comme la punaise de la molène) qu'aux prédateurs spécialistes (comme *P. persimilis*). J'espère que mes travaux inciteront d'autres chercheurs à développer le potentiel de cette approche.

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